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June 6 - 9, 2015
Glasgow, Scotland, United Kingdom

Abstracts

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Abstracts

European Society of Human Genetics

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1967 – 2017: 50th Anniversary of the ESHG
The European Human Genetics Conference 2017
Copenhagen, Denmark
May 27 – 30, 2017

European Human Genetics Conference 2018
Milan, Italy
June 16 – 19, 2018

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Abstracts highlighted with  are ESHG Poster Award Candidates.

PLENARY LECTURES

PL1.1
Chromosome conformation and long-distance gene regulation

N. Benabdallah[1], S. Bhatia[1], I. Williamson[1], **W. Bickmore[2];**
[1]MRC Human Genetics Unit, IGMM, University of Edinburgh, Edinburgh, United Kingdom, [2]Edinburgh, United Kingdom.

Long-range gene regulation first became apparent in mammalian genomes through Mendelian disease genetics associated with extreme phenotypes in human, and developmental genetics in the mouse. It is now appreciated that long-range enhancers - found as far away as 1 megabase from their target gene and located either in intergenic regions or in introns - are key in controlling the precise spatial and temporal expression of genes. Deletion, translocation or point mutations can abrogate the function of these elements in Rare Disease. However, the majority of human genetic variation associated with common and complex disease and quantitative traits also maps to intergenic regions that are likely the site of enhancers. Therefore, lessons learnt from studying enhancer dysfunction in rare disease will be important for an understanding of milder phenotypes.
It is hard to envisage how distant enhancers function if one only considers the genome as a linear DNA sequence. Rather, three-dimensional chromatin folding must play a fundamental role in enhancer-promoter communication. I will describe our work using different experimental approaches to investigate the three-dimensional folding of the mammalian genome at genetically defined long-range regulatory elements important in development.

PL1.2
Deciphering Developmental Disorders

M. Hurles;
Cambridge, United Kingdom.

No abstract received as per date of production. Check http://www.eshg.org/abstracts2015.0.html for possible updates.

PL1.3
Ribonucleotides embedded in genomic DNA

A. Jackson;
The University of Edinburgh, Edinburgh, United Kingdom.

Our identification of biallelic hypomorphic mutations in three RNase H2 genes in the neuroinflammatory disorder, Aicardi-Goutières syndrome led us to investigate enzyme complex they encode. We subsequently established this is an important genome surveillance enzyme that removes over 1,000,000 ribonucleotides embedded in the genomic DNA of every replicating mammalian cell. Such ribonucleotides represent the most common non-canonical nucleotides incorporated into the genome by replicative polymerases and are an important potential source of genome instability. Furthermore, we have now exploited embedded ribonucleotides as a tool to trace the respective contribution of DNA polymerases, developing the technique emRiboSeq, and use this to implicate replicative polymerases as a determinant of local nucleotide substitution rates at functionally important sites, shaping the mutational landscape of the genome.

PL2.1
De novo mutations in *PLXND1* and *REV3L* cause Möbius syndrome

L. Tomas Roca[1,2], *A. Tsaalbi-Shtylik[3], J. G. Jansen[3], M. K. Singh[4,5], J. A. Epstein[4], U. Altunoglu[6], H. Verzijl[7], L. Soria[1], E. van Beusekom[1], T. Roscioli[8], Z. Iqbal[1], C. Gilissen[1], A. Hoischen[9], A. P. M. de Brouwer[1], C. Erasmus[7], D. Schubert[10], H. Brunner[1,11], A. Pérez Aytés[12], F. Marin[2], P. Aroca Tejedor[2], H. Kayserili[6], A. Carta[13], N. de Wind[3], G. W. Padberg[7], H. van Bokhoven[1];*
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Introduction: Möbius syndrome (MBS) is a rare congenital neurological disorder characterized by facial and abducens nerve paralysis. Additional congenital anomalies are frequently associated. The etiology of this syndrome has been intensely debated and both teratogenic factors and genetic causes have been suggested. However, despite numerous clinical and molecular investigations since the description of the first patients in 1880 it has remained elusive.
Material and Methods: We hypothesized that de novo mutations contribute to the MBS phenotype and therefore performed exome sequencing in eight isolated MBS patients. All identified de novo variants were subsequently sequenced in a cohort of 103 MBS patients. The role of two candidate genes in MBS was addressed by morphological analysis of the knockout mouse brain.
Results: We report de novo mutations in two different genes PLXND1 and REV3L. Analysis of the Plxnd1 and Rev3l-knockout mice detected neuropathological findings similar to those found in MBS. Strikingly, both mouse models exhibited a reduced number of motoneurons in the facial motor nucleus, caused by defective neural migration in the Plxnd1-mutant mice or disrupted cellular proliferation in Rev3l-heterozygous mice. Conclusions: Our study establishs for the first time a genetic cause for MBS. We show morphological alterations in Plxnd1 and Rev3l mutant mouse models similar to MBS patients clinical features. Although PLXND1 and REV3L are involved in different pathways a protein defect of any of them result in a decrease of the number of motoneurons in the facial motor nucleus.

PL2.2
Beyond the ACMG 56: Parental choices and initial results from a comprehensive WGS-based search for predictive secondary variants in children

M. S. Meyn[1,2], *N. Monfared[1], C. Marshall[1], D. Merico[1], D. J. Stavropoulos[1,2], R. Z. Hayeems[1,2], M. Szego[3,2], R. Jobling[1], M. Gardia[2,1], G. D. Bader[2], M. Brudno[1,2], R. D. Cohn[1,2], R. Zlotnik-Shaul[1,2], C. Shuman[1,2], P. N. Ray[1,2], S. Bowdin[1,2];*
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To pilot the implementation of paediatric genomic medicine we developed the SickKids Genome Clinic, a multidisciplinary project supporting research into clinical whole genome sequencing (WGS). The Genome Clinic conducts diagnostic WGS for 150+ children/year who are undergoing genetic evaluations. With parents' permission, we search childrens' genomes for secondary medically-actionable variants (MAVs) in 2800+ disease genes listed in the NIH Clinical Genomic Database.
Of 321 families approached to date, 54% agreed to participate. 58% of participants chose to learn their child's secondary adult-onset MAVs. Among these parents, 68% decided to learn their own status for the same risk variants. Parents declining secondary variants were most concerned about psychological burdens and/or insurance discrimination.
Bioinformatics analysis of the first 80 patient genomes yielded 2382 candidate variants in 1117 genes that then underwent manual assessment (~30 variants/genome.) While ~15% were novel, the large majority of candidates were listed in HGMD or ClinVar. Most variants listed in HGMD as disease-causing failed manual assessment. E.g., >80% of variants found in the 56 ACMG genes and listed in HGMD as disease causing were rejected due to inadequate evidence of pathogenicity.
We identified 7 reportable variants in the 56 ACMG genes in our first 80 patients (~9%). By expanding our search to 2800+ genes we are finding secondary MAVs in >25% of children, ~40% of which lie in genes for adult onset disorders. We conclude that parental opinions vary widely regarding return of secondary variants, children may harbour more secondary MAVs than adults, and disease prevalence and imperfect variant interpretation constrain the number of reportable secondary MAVs.

PL2.3
Spotlight on the pathogenesis of Kabuki syndrome

N. Bögershausen[1], I. Tsai[2], E. Pohl[1], P. Simsek Kiper[3], F. Beleggia[1], F. E. Percin[4], K. Keupp[1], A. Matchan[5], E. Milz[1], Y. Alanay[6], H. Kayserili[7], Y. Liu[1], S. Banka[8], A. Kranz[9], M. Zenker[10], D. Wieczorek[11], N. Elcioglu[12], P. Prontera[13], S. Lyonnet[14], T. Meitinger[15], F. Stewart[9], D. Donnai[8], T. M. Strom[15], K. Boduroglu[3], G. Yigit[1], Y. Li[1], N. Katsanis[16], **B. Wollnik[1];**
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Italy, [14]Université Paris Descartes-Sorbonne Paris Cité, Institut Imagine, INSERM U781, Hôpital Necker-Enfants Malades, Paris, France, [15]Institute of Human Genetics, Helmholtz Zentrum München, Neuherberg, Germany, [16]Center for Human Disease Modeling and Department of Cell Biology, Duke University, Durham, NC, United States.

Kabuki syndrome (KS) is a genetic disorder characterized by developmental delay and multiple congenital anomalies. KS is caused primarily by mutations in *KMT2D (MLL2)*, a methyltransferase that promotes active transcription and, more rarely, in *KDM6A*, a demethylase of the KMT2D complex. Although the functions of chromatin modifying proteins have been well studied, the physiological systems regulated by them are largely unknown. Using whole exome sequencing we found a mutation converted to homozygosity by uniparental isodisomy (UPD) in *RAP1A* in a patient with KS, and a *de novo* dominant mutation in the closely related gene *RAP1B* in a second patient. We show that suppression of each of *RAP1* (*RAP1A* and *RAP1B*), *KMT2D*, and *KDM6A* leads to defective convergent-extension (CE) movements and to context-dependent MAPK signaling dysfunction. We further show that *RAP1* interacts genetically with *KMT2D* and that *RAP1B* expression is downregulated in both *kmt2d* morphant zebrafish (zf) embryos and KMT2D deficient patient cells. Regarding the frequent skeletal abnormalities in KS, we demonstrate that depletion of KS genes affects the layout of the pharyngeal skeleton in zf by disturbed F-actin polymerization and cell-cell intercalation. Interestingly, the CE and skeletal defects could be rescued *in vivo* by a small molecule MAPK inhibitor. Taken together, this study (i) identifies *RAP1A* and *RAP1B* as two novel genes for KS, (ii) reveals the first evidence that defective MEK-ERK signaling is a common molecular driver for KS, (iii) suggests that KS is a member of the RASopathy spectrum, and (iv) provides a potential direction for treatment design.

Grants

BMBF: 01GM1211A, 01GM1109C, 01GM1211B; NIH: R01DK075972, P50DK096415.

PL2.4

Disruptions of topological chromatin domains cause pathogenic rewiring of gene-enhancer interactions

D. G. Lupiáñez[1], *K. Kraft[1], V. Heinrich[2], P. Krawitz[1,2], F. Brancati[3], E. Klopocki[4], D. Horn[5], H. Kayserili[6], J. Opitz[7], R. Laxova[7], F. Santos-Simarro[8], B. Gilbert-Dussardier8[9], L. Wittler[1], M. Borschiwer[1], S. Haas[1], M. Osterwalder[10], M. Franke[1], B. Timmermann[1], J. Hecht[1,11], M. Spielmann[1,5,11], A. Visel[10,12,13], S. Mundlos[1,5,11];*

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Mammalian genomes are organized into megabase-scale topologically associated domains (TADs) that have been proposed to partition the genome into large regulatory units. Here we demonstrate that the disruption of TAD structure can cause rewiring of functional interactions between genes and distant-acting enhancers, resulting in pathogenic phenotypes in humans and mice. We show that distinct limb malformations in human patients are caused by deletions, inversions or duplications altering the structure of the extended WNT6/IHH/EPHA4/PAX3 locus. To examine these variants in detail, we adapted CRISPR genome editing to generate mice with corresponding large rearrangements. 4C-seq in mouse limb tissue and patient-derived fibroblasts showed that the structural changes result in ectopic interactions between promoters and non-coding DNA across adjacent TADs. On the disease alleles, a cluster of limb enhancers normally associated with Epha4 is misplaced relative to TAD boundaries, causing it to interact with and drive ectopic limb expression of Wnt6, Ihh or Pax3, respectively. Taken together, our results demonstrate the critical importance of TADs for the functional orchestration of the genome in vivo and support their utility in predicting the pathogenicity of human structural variants.

PL2.5

A germline homozygous loss-of-function mutation in the base excision repair gene NTHL1 causes adenomatous polyposis and colorectal cancer

R. D. A. Weren[1], *M. J. L. Ligtenberg[1], C. M. Kets[1], R. M. de Voer[1], E. T. P. Verwiel[1], L. Spruijt[1], W. A. G. van Zelst-Stams[1], M. C. Jongmans[1], C. Gilissen[1], J. Y. Hehir-Kwa[1], A. Hoischen[1], J. Shendure[2], E. A. Boyle[2], E. J. Kamping[1], I. D. Nagtegaal[1], B. B. J. Tops[1], F. M. Nagengast[1], A. Geurts van Kessel[1], J. H. J. M. van Krieken[1], R. P. Kuiper[1], N. Hoogerbrugge[1];*

[1]Radboud university medical center, Nijmegen, Netherlands, [2]University of Washington, Seattle, WA, United States.

Introduction: Patients diagnosed with adenomatous polyposis, i.e., the constitutive development of multiple colorectal adenomatous polyps, are at an increased risk to develop colorectal cancer (CRC). Currently, two adenomatous polyposis-associated syndromes are known: familial adenomatous polyposis (FAP) and *MUTYH*-associated polyposis (MAP). Whereas monoallelic *APC* germline mutations underlie FAP, MAP is caused by biallelic germline mutations in the base excision repair (BER) gene *MUTYH*. No other causative genes have been identified and, therefore, a considerable fraction of adenomatous polyposis patients remains unexplained

Materials and Methods: Whole-exome sequencing was applied to germline DNA derived from 51 individuals (from 48 families) with multiple colonic adenomas. After candidate gene selection, co-segregation analyses were performed. The somatic mutation spectrum of carcinomas and adenomas was determined by sequencing of 409 cancer-related genes and by targeted deep-sequencing of the *APC* gene, respectively.

Results: We identified a homozygous germline nonsense mutation in the BER gene *NTHL1* in seven affected individuals from three unrelated families. All three families showed recessive inheritance of the adenomatous polyposis phenotype, which was consistently and exclusively encountered in homozygous carriers, indicating a high-penetrant predisposing effect. In controls, this germline mutation was only found in a heterozygous state (MAF 0.0036, n=2,329). Sequence analysis of carcinomas and adenomas from different affected homozygous carriers revealed a non-hypermutated profile enriched for C-to-T transitions, in line with a germline BER defect.

Conclusions: We show for the first time that a homozygous loss-of-function germline mutation in the *NTHL1* gene predisposes to a novel subtype of BER-associated adenomatous polyposis and CRC.

PL2.6

The genetic handicap principle: a severely deleterious mutation can be tolerated if the genome-wide mutation load is sufficiently low

K. Popadin[1], *S. Peischl[2], R. Sailani[3], A. Letourneau[1], F. Santoni[1], M. Garieri[1], S. Nikolaev[1], D. Meyer[4], L. Excoffier[2], S. Antonarakis[1];*

[1]Department of Genetic Medicine and Development, Geneva, Switzerland, [2]Institute of Ecology and Evolution, Bern, Switzerland, [3]Stanford School of Medicine, Stanford, CA, United States, [4]University of Sao Paulo, Sao Paolo, Brazil.

Every human genome harbors hundreds of slightly-deleterious mutations. Even though most of these mutations have each a small effect, their cumulative effect (mutation load) could substantially reduce the fitness of an individual. Here we investigate an interaction between a severely deleterious mutation and the mutation load, formulating the genetic handicap principle: an individual bearing a severely deleterious variant (genetic handicap) is viable only if the genome-wide mutation load is sufficiently low. We develop a population-genetic model, which predicts that live-born individuals carrying a handicap mutation (causing, for example, 50-90% probability of miscarriage) show a reduction (5-20%) in their number of slightly-deleterious mutations as compared to controls. To test this prediction, we used data on trisomy of chromosome 21 (T21), a frequent chromosomal abnormality in humans which is associated with high miscarriage rates. Three evidences support the view that live-born T21 individuals have lower mutation load than the euploid population: (i) there is a deficit of homozygotes for rare derived alleles in the T21 cohort (N=338); (ii) the T21 cohort shows on chromosome 21 a deficit of gain-of-expression and an excess of loss-of-expression regulatory variants that can partially compensate the effect of trisomy; (iii) inter-individual whole-transcriptome variation in fibroblasts of the live-born T21 individuals (N=8) is lower than it is in the control cohort (N=8), reflecting potential selection against T21 fetuses with non-optimal pattern of expression. We conclude that the negative fitness consequences of severe mutations such as trisomy can be partially compensated by a reduced mutation load.

PL3.5

Should clinical geneticists have their genome sequenced during their training? Of course not!

Gijs W. Santen;
Leiden, Netherlands

Even asking the question whether clinical geneticists should have their genome sequenced would seem odd to any medical doctor: indeed, do we ask of gastroenterologists to undergo a colonoscopy during their training? Do anesthesiologist undergo anesthesia? Do radiologists undergo total-body MRI's? However, there is an exception: psychoanalysts do have to undergo

a 3-year 'training analysis' during their training. One can imagine that optimal psychoanalysis does depend on having an in-depth knowledge of your own psychological complexions and reactions to those of others. So the real question is perhaps whether a clinical geneticist is more comparable to a surgeon or a psychoanalysist. Does it help counseling a patient when you have in-depth knowledge of your own genome and the process that got you this knowledge?

An argument in favor of asking geneticists to sequence their own genome would be that personal understanding of the process that patients go through will improve counseling skill. However, there are important differences between genome sequencing of patients and healthy individuals, such as ourselves. In patients, we know that genome sequencing can be an efficient method to determine the genetic cause of an illness, which has implications for the patients themselves and their family. Indeed, that is the whole point! Yes, there is a risk of incidental findings but for most patients the risk-benefit ratio is acceptable. This is a different prospect than to ask a healthy individual to have their genome sequenced: what is the benefit that can be expected? To illustrate this, I have asked my Dutch peers who would have their genome sequenced, and the response was overwhelmingly negative. I think this reflects that we realize that at this point in time not much can be learned from the genome that would have major implications on our health, in the absence of symptoms in ourselves and a positive family history.

There are also practical objections that could be raised. First of all: the law. In the Netherlands at least, offering screening is bound to very strict laws and therefore sequencing a healthy person's genome might well be considered illegal! Cost is another one, but with sharply decreasing genome prices this may not be the most important hurdle. However, the data would have to be interpreted as well, and psychosocial counseling should be available for the geneticists who undergo sequencing. And who would analyze the data? Can I ask my dear colleagues to look at my genome data but to withhold information on late-onset untreatable disorders? And what if they find unclassified variants in colon cancer genes? 'How are you doing?' coming from the person who analyzed your data will never sound the same again!

In conclusion, although I do not believe all geneticists should have their genome sequenced during their training, I do think it might be interesting to start a pilot to offer this to a few of us, monitor the consequences and the costs. But I am not sure I would volunteer…

PL4.1
The neurexin enigma - from synapse formation to schizophrenia

T. Südhof;
Stanford, CA, United States.

No abstract received as per date of production. Check http://www.eshg.org/abstracts2015.0.html for possible updates.

PL5.1
ESHG Award Lecture

S. Pääbo;
Leipzig, Germany.

No abstract received as per date of production. Check http://www.eshg.org/abstracts2015.0.html for possible updates.

SYMPOSIA

S01.1
Integrative analysis of 80,000 whole exome sequencing and the Human Knock-out Project

D. McArthur;
Boston, MA, United States.

No abstract received as per date of production. Check http://www.eshg.org/abstracts2015.0.html for possible updates.

S01.2
Identification of a large set of rare complete human knockouts

P. Sulem;
Reykjavik, Iceland.

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S01.3
SISu project: 200,000 near complete sequences of Finns

A. Palotie;
Helsinki, Finland.

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S02.1
Whole genome sequencing in newborn screening? A Statement on the continued importance of targeted approaches in newborn screening programmes

H. C. Howard[1], *B. M. Knoppers*[2], *M. C. Cornel*[3], *E. Wright Clayton*[4], *K. Sénécal*[5], *P. Borry*[6], *European Society of Human Genetics, the P3G International Paediatric Platform, the Human Genome Organisation, the PHG Foundation;*
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The advent and refinement of sequencing technologies has resulted in a decrease in both the cost and time needed to generate data on the entire sequence of the human genome. This has increased the accessibility of using whole-genome sequencing and whole-exome sequencing approaches for analysis in both the research and clinical contexts. The expectation is that more services based on these and other high-throughput technologies will become available to patients and the wider population. Some authors predict that sequencing will be performed once in a lifetime, namely, shortly after birth. The Public and Professional Policy Committee of the European Society of Human Genetics, the Human Genome Organisation Committee on Ethics, Law and Society, the PHG Foundation and the P3G International Paediatric Platform address herein the important issues and challenges surrounding the potential use of sequencing technologies in publicly funded newborn screening (NBS) programmes. This statement presents the relevant issues and culminates in a set of recommendations to help inform and guide scientists and clinicians, as well as policy makers regarding the necessary considerations for the use of genome-sequencing technologies and approaches in NBS programmes. The primary objective of NBS should be the targeted analysis and identification of gene variants conferring a high risk of preventable or treatable conditions, for which treatment has to start in the newborn period or in early childhood.

S02.2
The 2014 ASHG Statement on Genetic testing in Children and Adolescence

J. Botkin;
Salt Lake City, UT, United States.

No abstract received as per date of production. Check http://www.eshg.org/abstracts2015.0.html for possible updates.

S02.3
Carrier testing in children and adolescents
S. A. Metcalfe;
Murdoch Childrens Research Institute and The University of Melbourne, Parkville Victoria, Australia.

There are a number of circumstances in which a child or adolescent may be identified, either unintentionally or intentionally, as a carrier for a recessive condition. These are: following newborn screening (NBS) when the test also reveals carrier results; as a secondary or incidental finding from investigations into an existing unrelated condition; cascade testing when another family member, especially a sibling, is identified with a genetic condition (or as a carrier through NBS); as a result of prenatal testing by carrier parents; population-based or ethnicity-targeted carrier screening of adolescents, most typically in high school. Since the purpose of carrier testing is to provide information for future reproductive planning, guidelines have traditionally recommended against carrier genetic testing of minors, yet testing in each of the above circumstances has occurred and, in some instances, for many years.

Arguments against carrier testing include: not being in the best interest of the child because it denies the child's future autonomy and removes their right to decide testing for themselves; the potential for psychological, emotional and social harms, such as stigma and discrimination; possibility for misunderstanding the meaning of carrier status; uncertainty whether testing at a young age would result in future reproductive behaviour. On the other hand, many parents have indicated they would want to know their child's carrier status and arguments in favour acknowledge that parents make health care decisions regularly for their child, and that the child's interest is inevitably intertwined with those of the parents and the family. Knowledge of a child's carrier status from newborn screening could be used by parents for their own future reproductive decisions. Learning carrier status as a young child allows them to adapt to the information and, as an older child, the information can increase reproductive options before they are sexually active. Proponents have also commented that there is a lack of evidence of harm to the child while others have called for more empirical research.

These arguments will be further elaborated and research will be highlighted that has examined the ethical and psychosocial aspects of carrier testing in children and adolescents in the various circumstances described above.

S03.1
Heritable germline epimutations in humans
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Essen, Germany.

Phenotypic variation results from genetic variation, epigenetic variation, environmental variation and cultural variation. While it is well established that we inherit our genes, our environment and our culture from our parents, it is less clear whether we also inherit epigenetic information. There is good evidence for epigenetic programming of the fetus by the maternal environment (mainly maternal glucose and hormone levels), but the transmission of epigenetic information through germ cells is a matter of debate. Epigenetic inheritance has been observed in plants and lower animals, but in mammals it faces two barriers: 1. the early separation of the soma and germ line (Weismann barrier) and 2. the epigenetic reprogramming of the genome during germ cell development and during early embryogenesis. Studies on epigenetic inheritance are hampered by the problem to distinguish epigenetic variants that have been transmitted through the germ line from epigenetic variants that arise anew in each generation as a consequence of inherited genetic, environmental or cultural variants. In fact, several reports on heritable germline epimutations have failed to recognize the presence of an underlying genetic mutation. On the other hand, it has been shown in rare cases that epigenetic states can - by accident - survive reprogramming in the germline. In these cases heritable epigenetic variation is most probably random and not adaptive. Nevertheless, it can be subject of selection.

S03.2
Maternal obesity during pregnancy and offspring later life disease
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One in five women in the UK is obese (body mass index BMI>30kg/m2) at antenatal booking. Maternal obesity is associated with complications for the mother including increased risk of developing gestational diabetes, pre-eclampsia and need for caesarean section. For the offspring short term complications include risk of macrosomia and need for admission to the neonatal unit. It is now apparent that the effects of maternal obesity for the offspring extend beyond the neonatal period with increased risk of obesity in childhood, adolescence and adult life. In addition there are risks of metabolic disturbance in childhood and young adulthood including glucose intolerance, hypertension and dyslipidaemia. In a record-linkage study we demonstrated that maternal obesity is associated with increased risk of premature mortality and hospital admissions for cardiovascular events in her adult offspring. Animal models suggest the adverse effects of maternal obesity on offspring outcomes are 'programmed' in utero. To investigate underlying mechanisms we have been carrying out a case-control study of very severely obese pregnant women (BMI>40kg/m2) vs. normal weight controls. We characterise maternal weight, body composition, and metabolic profiles through pregnancy and study infant growth and development at birth and 3 and 6 months. Placenta and cord blood are collected at birth and we are comparing placental function and epigenetic modifications of candidate genes in the lean and obese women. Our findings suggest early interventions to improve weight and diet in obese pregnant women are urgently needed. We have tested one such intervention in a randomised controlled trial in obese pregnant women using the insulin sensitiser metformin vs placebo with the aim of improving birth outcome.

S03.3
Regional activation of the cancer genome by long range epigenetic remodelling
S. Clark;
Sydney, Australia.

No abstract received as per date of production. Check http://www.eshg.org/abstracts2015.0.html for possible updates.

S04.1
Spliceosome biology: Understanding causes and consequences of splicing mutations
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Removal of noncoding intron sequences from the primary transcripts by the spliceosome is an essential step in eukaryotic gene expression pathway. Spliceosome is a molecular machine composed of five small nuclear RNAs (snRNAs) and numerous integral protein components. Human cells contain two separate spliceosomes, called as U2- and U12-dependent spliceosomes. U2-type spliceosome excises 99.5% of all introns in humans, while the U12-dependent spliceosome concentrates of a highly conserved subset of introns (termed as U12-type introns) that are found from approximately 700-800 genes in humans.

In addition to the essential role in generating functional mRNA molecules, the pre-mRNA splicing reaction serves also an important regulatory function. In a process called alternative splicing multiple mRNA isoforms are formed from a single gene. Alternative splicing is regulated through a number of non-spliceosomal protein components that can bind to regulatory sequences present in exon and intron sequences. Together with RNA secondary structure and chromatin modifications this leads to formation multiple mRNA species from a single gene that increases protein diversity but also provide important regulatory functions through regulating the stability of mRNA molecules.

A large number of human diseases are caused by defects in the pre-mRNA splicing process. Most common are mutations in either the splices site sequences located at intron-exon boundaries, or in the regulatory elements (splicing enhancers or suppressors) located in the exons or introns. Such mutations result in defects in pre-mRNA splicing that affect single gene only. In contrast, mutations in the components of the pre-mRNA splicing machinery lead to complex pleiotropic effects and affect the pre-mRNA processing in large number genes.

In my presentation I will describe the basic mechanism of intron recognition and splicing process. I will use the human disease causing mutations in the U12-dependent spliceosome to illustrate the effects that splicing machinery mutations have on pre-mRNA processing.

S04.2
Spliceosome and development in human
J. Amiel;
Institut Imagine, Paris, France.

Somatic and germline mutations of the spliceosomal machinery have first been described in cancers and retinitis pigmentosa. More recently, mutations in several genes involved in spliceosomal function or linked aspects of mRNA processing have been identified in human disorders with specific craniofacial malformations. As these gene products take part in widespread

cellular events, the phenotypic restriction is surprising and not yet fully understood. The syndromes and the pathophysiological hypotheses will be reviewed.

S04.3
Spliceosome and cancer

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The spliceosome machinery is a multi-subunit protein complex, responsible for mRNA processing. Recently, somatic mutations of genes encoding spliceosome components, such as SF3B1, U2AF1 and SRSF2, were discovered in haematological malignancies including myelodysplastic syndrome (MDS) and chronic lymphocytic leukemia (CLL).
While genetic lesions of the spliceosome are initiating events in MDS, they occur during disease progression in CLL. In acute myeloid leukemia (AML) spliceosome mutations seem to be rare and associated mostly with AML secondary to MDS, however, in the rare subgroup of AML with trisomy 13 the frequency of spliceosome alterations is strikingly high, with 81% of the patients habouring SRSF2 mutations. Of note, the mutational hotspot in SRSF2 is not captured efficiently by standard exome enrichment protocols. Thus, the burden of SRSF2 mutations might be underestimated.
Spliceosome mutations may alter gene expression or function by aberrant splicing, which could result in imbalance between alternatively spliced protein isoforms, non-physiological transcripts or haploinsufficiency due to nonsense-mediated RNA decay.
The exact mechanism of spliceosome dysfunction and the consequences for critical downstream genes during the onset and progression of neoplasia remain unclear.
Pharmacological inhibition of the spliceosome is currently being tested in early stage clinical trials opening up potential therapeutic applications.

S05.1
Using XIST to Silence Trisomy 21: Implications for Cell and Chromosome Therapy

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Down syndrome, also known as trisomy 21, is the leading genetic cause of intellectual disabilities, occurring in 1 out of 1000 live births. The millions of Down syndrome patients across the world also face multiple other health issues, including congenital cardiac defects, high incidence of Early-onset Alzheimer disease, and hematopoietic disorders. Given that Down syndrome is caused by an extra copy of chromosome 21 that involves over-dosage of 400 genes across a whole chromosome, it precludes any possibility of a genetic therapy. Our lab has long studied the natural dosage compensation mechanism for X chromosome inactivation. To "dosage compensate" X-linked genes between females and males, the X-linked *XIST* gene produces a large non-coding RNA that silences one of the two X chromosomes in female cells. The initial motivation of this study was to translate the natural mechanisms of X chromosome inactivation into chromosome therapy for Down syndrome. Using zinc finger nuclease technology, we have successfully inserted a large *XIST* transgene into Chromosome 21 in Down syndrome iPS cells, which results in chromosome-wide transcriptional silencing of the extra Chromosome 21. Remarkably, deficits in proliferation and neural growth are rapidly reversed upon silencing one chromosome 21. Successful trisomy silencing *in vitro* surmounts the major first step towards potential development of "*chromosome therapy*" for Down syndrome. The human iPSC-based trisomy correction system we established opens a unique opportunity to identify molecular networks driving different aspects of pathogenesis and study transplantation therapies for Down syndrome.

S05.2
Having developed an accurate noninvasive prenatal test for aneuploidies- What else can we work on?

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The discovery of the presence of fetal DNA in the plasma of pregnant women has revolutionized the landscape of prenatal testing. In particular, the noninvasive prenatal test for chromosomal aneuploidies through sequencing maternal plasma DNA has been rapidly adopted throughout the world over the last 5 years. It is estimated that over 1 million noninvasive prenatal tests for chromosomal aneuploidies have been performed. The principle of this test is based on the detection of the increased amount of DNA released from the extra copy of the aneuploid chromosome of the fetus. The chromosome dosage can be accurately determined by counting the number of sequenced plasma DNA molecules mapping to the individual chromosomes. The sensitivities and specificities of this approach are remarkable for detecting trisomies 13, 18 and 21 in large-scale studies. However, this approach is recommended as a screening test and further confirmation of positive results by testing of fetal tissues obtained invasive procedures is recommended. In this lecture, the following questions as well as potential solutions will be discussed:
1. Will this approach be accurate enough to serve as a diagnostic test instead of a screening test?
2. Is it possible to differentiate chromosome aneuploidies coming from the mother and from the fetus?

S05.3
Status and outcome of randomized trials for aneuploidy screening preimplantation embryos

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The primary aim of aneuploidy screening preimplantation embryos is to optimize outcomes in couples undergoing infertility treatment. Since the first *in-vitro* fertilization (IVF) cycle performed in 1978, it is estimated that over 5 million babies have been born following IVF. However, only about one third of IVF cycles result in the birth of a baby. Selection of IVF embryos most likely to implant is traditionally based on embryo morphology, although this is relatively imprecise. Based on observations that aneuploidies in clinical miscarriages increase with advanced maternal age (AMA), that aneuploidies are found in preimplantation IVF embryos and that most aneuploidies are incompatible with life, a rationale was developed to improve IVF pregnancy rates through selecting chromosomally normal embryos for embryo transfer. This procedure, usually called Preimplantation Genetic Screening (PGS), was initially applied 20 years ago for AMA, using FISH to analyze select chromosomes in single blastomeres biopsied from 3-day embryos (PGS version 1, PGS-1). Indications for PGS were subsequently widened to couples undergoing IVF for repeated implantation failure, spontaneous abortions and severe male infertility. However, following 10 years of clinical application, evidence from randomized trials did not support a clear benefit of PGS-1 (FISH on blastomeres). Reasons likely include high rates of mosaicism in day-3 preimplantation embryos, the inability of FISH to analyse all chromosomes in a single cell and possibly a negative impact of blastomere biopsy itself. Subsequent PGS strategies use 24-chromosome array-CGH analysis on biopsied polar-bodies or trophectoderm biopsies from blastocyst-stage embryos (PGS-2). Good randomized control trials are challenging, involving stringent patient recruitment, satisfactory patient numbers, monitoring all procedures/protocols, time and expense. The few on-going or recently published randomized trials have not yet clarified the optimum strategy and specific benefits (if any) of aneuploidy screening preimplantation embryos. Results of recent randomized trials will be presented.

S06.1
DECIPHER

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No abstract received as per date of production. Check http://www.eshg.org/abstracts2015.0.html for possible updates.

S06.2
„Sharing Data in Cancer Genomics; Lessons from the International Cancer Genome Consortium"

S. Grimmond;
Glasgow, United Kingdom.

No abstract received as per date of production. Check http://www.eshg.org/abstracts2015.0.html for possible updates.

S06.3
The Challenge of the Global Variome
J. Burn;
Newcastle upon Tyne, United Kingdom.

No abstract received as per date of production. Check http://www.eshg.org/abstracts2015.0.html for possible updates.

S07.1
The International Mouse Phenotyping Consortium: New insights into the genetic and molecular bases of disease
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A major challenge facing mammalian genetics over the next decade is the systematic and comprehensive annotation of mammalian gene function. As part of the International Knockout Mouse Consortium, several programmes are ongoing to generate conditional mutants for all mouse genes. An even greater challenge will be the determination of phenotypic outcomes for each mutation and the identification of disease models. The International Mouse Phenotyping Consortium (IMPC, www.impc.org) will undertake the development of a comprehensive Catalogue of Mammalian Gene Function and proposes to build on the several pilot programmes that have explored the feasibility of large-scale mouse phenotyping, such as the EUMODIC programme. The IMPC incorporates 16 major mouse centres around the world that will undertake mouse production and phenotyping. The IMPC envisages two phases to its programme: Phase 1, 2011-2016, is already well underway and is carrying out the phenotyping of around 5000 mouse lines; and Phase 2 from 2016-2021 which will undertake the analysis of 15,000 mouse lines. IMPC centres operate a core, standardised, broad-based adult phenotyping pipeline encompassing the major biological and disease systems, including gross pathology and tissue collection as a mandatory requirement. Many centres have also begun to employ a standardised embryonic phenotyping pipeline to analyse the many homozygous lethals, incorporating an assessment of time of lethality and morphological defects. In addition, lacZ expression data is being collected for adult organs and E12.5 embryos. All data from each production and phenotyping centre is uploaded to a central Data Coordination Centre (DCC), and following QC and analysis is archived and disseminated to the wider biomedical sciences community along with appropriate annotation tools. In the first 3 years of the programme, over 7000 ES cell lines have been injected, close to 4000 mouse mutant lines generated and phenotype data from nearly 2000 mutants collected at the DCC. We will describe many new insights into the genetic and molecular bases of disease, report the generation of numerous novel disease models, and elaborate a fundamental appraisal of the pleiotropic landscape of mammalian gene function.

S07.2
Investigating genetic diseases with intellectual disability in the mouse
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Intellectual disability (ID) involved impairment of mental abilities that impacts adaptive functioning in the conceptual, the social or the practical domain. ID can occur during the developmental period and is defined by an intellectual quotient below 70. Several genetic conditions, including Down syndrome, copy number variants, and more than 500 genes, have been now associated with ID. To better understand the physiopathology of the different causes of ID, we generated a large series of mouse mutants for ID genes involved in different functions such as synaptic transmission or nuclear regulation, during the GENCODYS program (www.gencodys.eu). Here we will report the characterization of several ID mouse models using standardized behavioural and cognitive paradigms. We also took advantage of the International Mouse phenotyping resource to get additional information on the phenotypes of the ID models. Based on the new series of models and further studies, several ID genes were found inducing key defects in the mouse confirming, even for some challenged candidates, their involvement in ID. In addition we went further investigating more complex genetic conditions, such as copy number variant, and we were able to identify genes and their impact on the behaviour and cognition. The data generated are challenging our current knowledge on the role of ID genes, suggesting common outcome for various genetic conditions. Such studies offer perspectives for a better understanding of the ID and how the cognition and behaviour is affected in human.

S07.3
Deciphering the genetic and epigenetic role in metabolic diseases
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S08.1
Constitutional and somatic variations in telomerase reverse transcriptase and human cancer
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Human telomerase reverse transcriptase (TERT) encodes a rate limiting catalytic subunit of telomerase. We earlier described a disease segregating causal mutation at the -57 bp from ATG start site of the TERT gene in a large melanoma family with early on-set and severe form of the disease. Screening of the TERT core promoter in tumors from unrelated melanoma patients showed mutually exclusive mutations at the -124 and -146 bp from the ATG site at a frequency higher than any other mutation in melanoma (Horn et al. Science, 2013, 339:959-61). The familial and somatic mutations create de novo CCGGAA/T binding motifs for E-twenty six/ternary complex factors (Ets/TCF) transcription factors with consequent increase in TERT expression. The TERT promoter mutations have now been shown to be widespread in many cancer types and have been regularly associated with an increased gene expression and adverse forms of the disease. In bladder cancer, we demonstrated an association of the TERT promoter mutations with a poor patient survival and an increased disease recurrence (Rachakonda et al. PNAS 2013, 110:17426-31). While in glioma, TERT promoter mutations also associated with poor progression free and poor overall survival; in melanoma those mutations associated with parameters connected with poor outcome (Heidenreich et al. Oncotarget 2015; Heidenreich et al. Nature Communications 2014; 5:3401). Germline TERT promoter mutations in carriers result in an increased leukocyte telomere length; however, tumors with somatic TERT promoter mutations have shorter telomeres than tumors without mutations (Hosen et al. International Journal of Cancer 2015). Those findings have resulted in major conceptual advancements and have shown that tumor specific changes in a non-coding region can initiate cancer through change in gene expression. The effects of the promoter mutations on TERT transcription and telomere length emphasize the importance of telomere biology in cancer genesis and outcome.

S08.2
The role of telomeres in aging
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S08.3
Novel insights into the telomere syndromes
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Dyskeratosis Congenita (DC) is a heterogeneous multi-system syndrome exhibiting marked clinical and genetic heterogeneity. In its classical form it is characterised by mucocutaneous abnormalities (abnormal skin pigmentation, nail dystrophy, leucoplakia), bone marrow failure and a predisposition to malignancy. Bone marrow failure is the principal cause of mortality and patients display features of premature aging.
Studies over the last two decades have led to significant advances with 11 disease genes (*DKC1*, *TERC*, *TERT*, *NOP10*, *NHP2*, *TINF2*, *USB1*, *TCAB1*, *CTC1*, *RTEL1*, and *ACD*) having been characterized. Ten of these are important in telomere maintenance. DC is therefore principally a disease of defective telomere maintenance and patients usually have very short/ and or abnormal telomeres.
The genetic advances have also led to the unification of DC with a number of other disorders. This includes the severe multi-system disorders Hoyeraal-Hreidarsson and Revesz syndromes as well as a subset of patients with aplastic anaemia, myelodysplasia, leukaemia, liver disease and idiopathic pulmonary fibrosis. This wide spectrum of diseases ranging from classical DC to aplastic anaemia can be regarded as disorders of defective telomere maintenance - "the telomereopathies".

Some cases of DC still remain uncharacterized. Using whole exome sequencing we have identified novel biallelic mutations in the poly(A)-specific ribonuclease (*PARN*) gene, in families exhibiting severe DC. PARN is an exonuclease whose deadenylation activity in part controls mRNA stability and therefore regulation of a large number of genes. The mutations identified affect key domains within the protein and studies on patient cells show reduced deadenylation activity. This deficiency causes an early DNA damage response, cell cycle arrest and reduced cell viability upon UV treatment. Individuals with biallelic *PARN* mutations and cells that are depleted of PARN have reduced RNA levels for several key genes associated with telomere biology (*TERC*, *DKC1*, *RTEL1* and *TERF1*). They also possess critically short telomeres. Collectively, these results identify a role for PARN in telomere maintenance and demonstrate that it is a new disease-causing gene in a subset of cases with severe DC.

S09.1
The AML Genome(s)

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Acute myeloid leukemia (AML) is characterized by clinical, morphologic, genetic, genomic, and epigenomic heterogeneity. AML genomes are the most minimally mutated adult human neoplasms sequenced to date, with an average of only 13 genic somatic mutations (of which only 5 are in recurrently mutated AML genes). The complexity of the mutational associations is so great that it has not yet been possible to create a new classification scheme for all *de novo* AML patients based on the presence or absence of mutations alone.
Virtually all AML cases are clonally heterogeneous at presentation; this heterogeneity is influenced by treatment, and usually evolves at relapse. Nearly all de novo AML genomes have a single founding clone, and one or more subclones that are derived from it, or from another subclone. Although the genetic rules governing resistance and relapse are not yet clear, mutations that activate signaling pathways (e.g. *FLT3*, *KIT*, and *RAS* mutations, among others) are most often late mutations that occur in subclones, where they may provide a signal for explosive outgrowth. These mutations are usually cleared by induction therapy, while initiating mutations (e.g. in *DNMT3A* or *TET2*) are not.
Since we do not yet know the mutations that are relevant for resistance and/or relapse, and since the clonal architecture of the tumor at presentation may influence response and relapse, clinical sequencing will require not only an ability to identify the genes that are mutated in each sample, but also to an ability to define clonal architecture, and how subclones respond to induction therapy. The failure to clear all mutations after induction therapy significantly increases the risk of relapse, and reduces overall survival. Additional studies focusing on the epigenomes of AML cells will be required to fully understand the pathways and mechanisms that underlie the pathogenesis of this disease.

S09.2
Reconstruction of clonal composition in cancer

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Cells from a tumour are often not isogenic. The fraction of cancerous cells rather consists of a collection of subclones, with private and shared mutations, related by their joint evolutionary history. Such heterogeneity poses a challenge to cancer therapies. There is substantial evidence that it can underpin the emergence of resistance and so adversely affects treatment outcomes. Therefore, the ability to track subclonal dynamics and changes in clonal composition can inform therapy.
The challenge is that it is still not possible to sequence individual cells routinely to capture the full information about their genotype. Instead, short-read sequencing of cell populations is typically used in cancer genomics. Therefore it is necessary to use computational methods to reconstruct the subclonal lineages from sequenced samples.
Here we describe our work on the subclone reconstruction problem with applications to chronic lymphocytic leukaemia, colorectal cancer and experimental evolution.

S09.3
Genomic medicine to tailor cancer drugs

N. Normanno;
Naples, Italy.

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S10.1
Genomics and Hypertension

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Human primary or essential hypertension is a complex, polygenic trait with some 50% contribution from genes and environment. Richard Lifton and colleagues provided elegant dissection of several rare Mendelian forms of hypertension, exemplified by the glucocorticoid remediable aldosteronism and Liddle's syndrome. These discoveries illustrate that a single gene mutation can explain the entire pathogenesis of severe, early onset hypertension as well as dictating the best treatment.
The dissection of the much more common polygenic hypertension has proven much more difficult. Early studies used a single polymorphic marker such as the I/D polymorphism in the ACE gene and small numbers of cases and controls. Candidate gene studies have been largely non-informative and non-reproducible. These were followed by linkage studies, which used approximately 300 microsatellite markers distributed across the genome. These studies resulted in large peaks covering regions with 50-100 genes, with no easy way to quickly focus on a few genes of causal relevance. The real breakthrough came with the initiation of the genome wide association studies (GWAS) characterised by a much more thorough coverage of the genome with thousands single nucleotide polymorphisms (SNPs). Typically 500,000 - 2,5000,000 SNPs have been used for the big, collaborative GWAS for hypertension. These studies resulted in several "hits" or signals with a genome-wide significance and a high level of reproducibility between studies. These "hits" have been used successfully to calculate genetic risk scores for cardiovascular complications such as left ventricular hypertrophy, stroke and coronary artery disease. Intragenic signals, such as for example Uromodulin, are being used to examine new pathways for cardiovascular protection and possibly new targets for drug discovery.
The next steps in genomic medicine belong to a combination of the next generation sequencing (NGS) and its linkage with electronic health records, including preferably the real time clinical data, biochemistry, imaging, histology as well as longitudinal health outcomes. These modalities of stratified or precision medicine are ready for the prime time now.

S10.2
Ten Years Later : How The Pcsk9 Gene Discovery Affects the Diagnosis and Treatment of Hypercholesterolemia

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Paris, France.

No abstract received as per date of production. Check http://www.eshg.org/abstracts2015.0.html for possible updates.

S10.3
Genetic variation in APOC3, plasma triglycerides and risk of ischemic cardiovascular disease

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Background: High plasma levels of nonfasting triglycerides are associated with increased risk of ischemic cardiovascular disease. Whether lifelong low levels of nonfasting triglycerides, due to mutations in the gene for apolipoprotein C3 (APOC3), associate with reduced risk of ischemic cardiovascular disease in the general population is unknown. This is important, because APOC3 is a potential drug target for reducing residual cardiovascular risk.
Methods: Using 75,725 individuals from two general-population studies, we first tested whether low levels of nonfasting triglycerides were associated with reduced risk of ischemic vascular disease and ischemic heart disease. Second, we tested whether loss-of-function mutations in APOC3, which were associated with reduced levels of nonfasting triglycerides, were also associated with reduced risk of ischemic vascular disease and ischemic heart disease. During follow-up, 10,797 individuals developed ischemic vascular disease, of whom 7,557 developed ischemic heart disease.

Results: Individuals with nonfasting triglyceride levels less than 1.00 mmol/L had significantly less cardiovascular disease than those with levels of 4.00 mmol/L or higher (hazard ratio [HR] 0.43; 95% confidence interval [CI], 0.35-0.54 for ischemic vascular disease and HR 0.40; 95% CI, 0.31-0.52 for ischemic heart disease). Heterozygosity for loss-of-function mutations in APOC3 was associated with mean reductions in plasma nonfasting triglycerides of 44% (P=2x10-54). The cumulative incidence of ischemic vascular disease and ischemic heart disease was reduced in heterozygotes (P=0.009 and 0.05, respectively), with corresponding risk reductions of, respectively, 41% (HR: 0.59; 95% CI, 0.41-0.86; P=0.007) and 36% (HR: 0.64; 95% CI, 0.41-0.99; P=0.04).
Conclusions: Loss-of-function mutations in APOC3 are associated with low levels of triglycerides, and with reduced risk of ischemic cardiovascular disease. This suggests that APOC3 is an excellent drug target for reducing residual cardiovascular risk.

S11.1
Retrotransposons and human disease

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Long Interspersed Element-1 (LINE-1 or L1) is a family of active non-LTR retrotransposons that comprise around a fifth of our genome. Despite this substantial representation, an average human genome only contains 80-100 actively mobile or retrotransposition-competent L1s (RC-L1s). The mobility of RC-L1s continues to impact germline and somatic genomes and can lead to human genetic disease. L1 retrotransposition normally occurs by a mechanism known as target site-primed reverse transcription (TPRT), which requires both the L1-encoded endonuclease and reverse transcriptase activities. However, it has been reported that L1 can mobilize through an endonuclease-independent pathway (ENi), in which the element inserts at sites of DNA disrepair. Notably, recent data from our lab has demonstrated that the mobilization of L1 is de-regulated in Fanconi Anemia (FA) patients, including the ENi pathway. FA is a rare disease characterized by infant mortality and genomic instability, suggesting that de-regulated LINE-1 retrotransposition may affect genomic fluidity in FA patients.

S11.2
CNVs of noncoding cis-regulatory elements in human disease

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No abstract received as per date of production. Check http://www.eshg.org/abstracts2015.0.html for possible updates.

S11.3
A Novel Dicer1-miR328-Bace1 Signaling Axis Controls Ageing- and Obesity-Induced Brown Fat Dysfunction

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Activated brown adipose tissue (BAT) contributes to control of energy and glucose homeostasis in rodents and humans. Defining the cell-autonomous processes that underly BAT differentiation and activation may thus reveal novel therapeutic targets for obesity and type 2 diabetes mellitus intervention. Here we show that ageing- and obesity-associated demises in BAT function coincide with coordinate down-regulation of mature microRNAs in BAT in the presence of reduced expression of the microRNA processing enzyme Dicer1. To mimic this partial down-regulation of microRNA processing in obesity and ageing, we inactivated one allele of Dicer1 selectively in BAT of mice. BAT-selective heterozygosity of Dicer1 caused glucose intolerance in lean mice and aggravated diet-induced-obesity (DIO)-evoked deterioration of glucose homeostasis. Using combinatorial analyses of altered microRNA-expression in BAT during in vitro brown preadipocyte commitment and mouse models of premature ageing, longevity and DIO, we identified 23 microRNAs dysregulated among these conditions. Of these, we identified miR-328 as a novel regulator of BAT differentiation. miR-328 over-expression promotes BAT-differentiation and impairs muscle progenitor commitment, while reducing miR-328 expression blocks brown but not white adipocyte differentiation. We validated the ß-Secretase Bace1 as a target of miR-328, which is consequently over-expressed in BAT of obese and premature ageing mice. Reducing Bace1 expression enhances brown adipocyte, while impairing myogenic differentiation in vitro. In vivo small-molecule Bace1 inhibition in obese mice delayed DIO-induced weight gain, ameliorated obesity-associated deterioration of glucose metabolism and improved insulin sensitivity. Collectively, these experiments reveal reduced Dicer1-miR-328-Bace1 axis in presence of generalized impairment of microRNA processing in ageing and obesity as a novel determinant of ageing- and obesity associated decline in BAT function. This may define in vivo Bace1-inhibition as an innovative therapeutic approach to not only target age-related neurodegenerative diseases but at the same time improving age-related impairment of BAT-function and metabolism.

S12.1
Mitochondria in neurodegeneration

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S12.2
A localized autophagic filter prevents entry of mitochondria carrying pathogenic Opa1 mutations in retinal ganglion cell axons

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Optic Atrophy 1 (Opa1) is a multifunctional protein involved in mitochondrial fusion, apoptosis and metabolism, but how its mutations affect retinal ganglion cell (RGC) health and result in Autosomal Dominant Optic Atrophy (ADOA) remains unknown. Here we show that pathogenic Opa1 triggers a localized accumulation of autophagosomes that filters mitochondria out from RGC axons. Mitochondrial dysfunction caused by mutated Opa1 results in localized AMPK activation and downstream axonal hillock autophagosome accumulation. Pharmacological or genetic autophagy inhibition restores axonal mitochondrial entry and rescues RGCs from apoptosis caused by mutated Opa1. In vivo, genetic autophagy inhibition rescues visual loss caused by Opa1 ablation. Thus, localized mitochondria autophagy emerges as a mechanism of neuronal dysfunction.

S12.3
Gene therapy for mitochondrial neurogastrointestinal encephalomyopathy (MNGIE)

R. Martí;
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MNGIE (mitochondrial neurogastrointestinal encephalomyopathy) is a mitochondrial disease caused by mutations in the nuclear gene *TYMP*, encoding thymidine phosphorylase (TP). In recent years, knowledge gained from basic research on the biochemical mechanisms involved in this disorder has allowed us to design plausible therapy approaches. In MNGIE patients, TP dysfunction leads to systemic overload of the nucleosides thymidine and deoxyuridine, which results in alteration of the homeostasis of mitochondrial deoxyribonucleoside triphosphate (dNTP) pool. This imbalance interferes the correct replication of mitochondrial DNA (mtDNA). As a consequence, mtDNA depletion, multiple deletions and somatic point mutations occur in several tissues in patients, ultimately leading to mitochondrial dysfunction. As the clinical phenotype of MNGIE is the result of the toxic accumulation of thymidine and deoxyuridine, therapy approaches have focused on clearing the systemic overload of these nucleosides. First attempts to use hemodialysis failed to reduce nucleoside overload because of the high rate of endogenous production of these compounds by human metabolism. By contrast, hematopoetic stem cell transplantation restored nucleoside homeostasis in patients with successful engraftment, and led to slow clinical improvement. However, the high morbidity and mortality rates associated to the procedure encouraged us to find alternatives, and the most obvious one, gene therapy, has given very promising results in a murine model of the disease. Two different vectors carrying the human *TYMP* gene (a lentiviral vector transduced to hematopoietic stem cells, and an adeno-associated virus vector with targeted expression in liver) have been tested in a murine model of the disease. In both cases, successful and long-term stable expression of the transgene was achieved, resulting in permanent reduction of nucleoside overload *in vivo*. These results demonstrate that gene therapy is a feasible option for MNGIE patients; therefore, clinical trials should be implemented to investigate the safety and efficacy of this option.

S13.1

Nonsense suppression strategies to treat ocular malformations

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The extensive genetic and allelic heterogeneity observed with congenital eye diseases is a major barrier to the development of therapeutics. Leber congenital amaurosis is a typical example where more than 400 mutations have been identified in at least 19 different genes. Conversely, more than 600 mutations in a single gene (PAX6) cause aniridia. Developing treatments for each affected gene remains a significant challenge and would have to overcome the ethical and technical barriers associated with the delivery of a preventative prenatal treatment. To overcome these issues we have taken advantage of the observation that approximately 12% of all disease-causing mutations are nonsense mutations leading to premature stop codons. Our approach has therefore focussed on pharmacological strategies that specifically target nonsense mutations and are gene-independent. In our recent work we used systemic prenatal and postnatal nonsense suppression to correct the underlying genetic defects in choroideremia, ocular coloboma, aniridia, retinitis pigmentosa and most recently Leber congenital amaurosis. During these studies we developed a topical eye drop formulation (called START therapy) containing the nonsense suppression drug Ataluren that we tested in a mouse model of aniridia. Topical delivery not only reversed the malformation defects it also restored the electrical and behavioral responses of the retina. Further studies on the efficacy of this postnatal treatment have shown that the eye responds to changes in Pax6 dosage over a specific time window. These studies suggest that the eye retains significant developmental plasticity into the post-natal period and therefore postnatal therapeutic strategies delivered early in life could now be considered as potentially practical for some congenital eye abnormalities. Not only would topical drug delivery avoid the issue of systemic toxicity, but would likely lead to high compliance in young children. Furthermore, this approach would be relevant to new mutations that spontaneously occur without family history.

S13.2

Therapeutic targeting of the mTOR pathway

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Signalling through the mammalian (or mechanistic) target of rapamycin (mTOR) complex 1 (mTORC1) is hyperactivated in many sporadic cancers and in a family of inherited conditions such as tuberous sclerosis, PMSE syndrome and the DEPDC5-associated epilepsies that are characterised by variable combinations of hamartomas and other tumours, epilepsy and neurodevelopmental disorders. Rapamycin and its derivatives (the "rapalogs") inhibit mTORC1 and are being explored as therapies in these diverse clinical settings. Successful clinical trials have led to regulatory approvals in Europe and North America of Everolimus for the treatment of some sporadic cancers and tuberous sclerosis-associated renal and brain tumours. Promising results from phase II trials have led to current phase III trials in epilepsy and phase II trials are assessing efficacy and safety of rapamycin and rapalogs in relation to neurocognitive and neurodevelopmental deficits. Pre-clinical research and early phase trials are also exploring opportunities for targeting the signalling networks upstream and downstream of mTORC1 in sporadic cancers and tuberous sclerosis. In this talk I will review progress to date in translation to the clinic and discuss new therapeutic strategies that are in development.
J Sampson is Chief Investigator of the TRON trial which is funded significantly by Novartis Pharmaceuticals and has received modest lecture fees/Honoraria from Novartis Pharmaceuticals

S13.3

Efficient AAV gene therapy in cardiac and neurological murine models for Friedreich ataxia

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Friedreich's ataxia (FRDA), the most common autosomal recessive ataxia, is characterized by a sensory and spinocerebellar ataxia, hypertrophic cardiomyopathy and increase incidence of diabetes. FRDA is caused by reduced levels of frataxin (FXN), an essential mitochondrial protein involved in the biosynthesis of iron-sulfur (Fe-S) clusters. Impaired mitochondrial oxidative phosphorylation, bioenergetics imbalance, deficit of Fe-S cluster enzymes and mitochondrial iron overload occur in individuals with FRDA. To date there are not effective treatment for FRDA, and cardiac failure is the most common cuase of mortality in FRDA. We recently showed that adeno-associated virus (AAV) rh10 vector expressing human FXN injected intravenously not only prevented the onset of the cardiac disease in a faithful FRDA cardiac mouse model, but also, when administered at the time of heart failure, reversed rapidly and completely cardiac disease at the functional, cellular and molecular level in all treated animals. Our results also demonstrated the capacity of defective cardiomyocytes with severe energy failure and ultrastructure disorganization to be rapidly corrected and remodeled by gene therapy. These results established the primary proof-of-concept for developing gene therapy of FRDA cardiomyopathy. We are currently performing dose-response study in the mice to estimate the percentage of cardiomyocytes that need to be corrected to have a therapeutic effect. In addition, we have recently generated a novel mouse model that recapitulates faithfully the sensory ataxia associated to FRDA. These mouse models will be essential to dissect the pathophysiological pathway associated to the disease but also to test therapeutic approaches, including gene therapy.

S14.1

Chromatin organization and long-range control of gene expression

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Cell-type specific gene regulation is under the purview of enhancers. Great strides have been made recently to characterize and identify enhancers both genetically and epigenetically for multiple cell types and species, but efforts have just begun to functionally characterize these long-range control elements. Mapping interactions between enhancers and promoters, and understanding how the 3D landscape of the genome constrains such interactions is fundamental to our understanding of enhancer function. I will present recent findings related to 3D genome organization in mammalian cells, with a particular focus on how chromatin organization contributes to enhancer-mediated transcriptional regulation. I will describe higher-order organizational features that are observed at the level of both the whole chromosome and individual loci. I will highlight changes in genome organization that occur during the course of differentiation, and discuss the functional relationship between chromatin architecture and gene regulation. Taken together, mounting evidence now shows that the genome organization plays an essential role in orchestrating the lineage-specific gene expression programs through modulating long- range interactions between enhancers and target genes.

S14.2

Biological consequences of regulatory variation and disease

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Molecular phenotypes inform us about genetic and environmental effects on cellular and tissue state. The elucidation of the genetic basis of gene expression and other cellular phenotypes is highly informative for the impact of genetic variants in the cell and the subsequent consequences in the organism. In this talk I will discuss recent advances in key areas of the analysis of the genomics of gene expression and cellular phenotypes in human populations and multiple tissues and how this assists in the interpretation of regulatory networks and human disease variants. I will also discuss how the recent advances in next generation sequencing and functional genomics are informing us about the impact of regulatory variation in cancer. Finally, I will present some perspectives on how these developments are bringing us closer to the promise of personalized medicine.

S14.3

Mutations in regulatory domains in human disease

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One of the key discoveries of vertebrate genome sequencing projects was the unexpected amount of DNA that remained evolutionarily conserved under selective pressure (or even non conserved despite likely functional; see EnCode project). Two-thirds of it does not correspond to coding sequences (exons and UTRs), which have been named conserved non-coding sequences (CNCs) and represent a vast amount of DNA (> 3% of the human genome). Interestingly, enrichment for CNCs has been demonstrated within gene deserts nearest to physically isolated genes known or suspected to be important developmental regulators. It has thus been suggested that, in these cases, CNCs may represent regulatory elements (enhancers or suppressors) necessary for the correct spatiotemporal expression of these genes needed

for embryonic development, and acting as modular, sometimes combinatorial, tissue-specific enhancers of gene transcription.
In that context, we will discuss a number of findings, following the seminal discovery of long-distance genomic alterations altering the expression of the SHH (Sonic Hedgehog) gene. Recent examples involve mutation of non-coding RNA genes (such as the deletions observed at the mir17-92 cluster in Feingold syndrome), but also regulatory DNA alterations that we will discuss as:
- Enhancer variants located within or close to a gene, such as a genomic variant in a highly conserved sequence located in a non-coding region of the RET gene, altering the binding of a transcription factor expressed in neural crest cell precursors to the enteric nervous system, which predispose to Hirschsprung disease.
- Long-distance disruption of CNCs, whatever their function, such as those observed on both side of the SOX9 gene coding sequences in either Pierre Robin sequence (PRS), a common orofacial cleft anomaly with mandibular hypoplasia, or isolated disorders of sex determination (DSDs). In these cases, the disruption of distant tissue-specific regulatory elements, required for the normal development of either the mandibula or the gonads, perturbs embryonic expression of SOX9 and could account for the PRS or DSD phenotypes respectively, as these evolutionarily constrained regions may be disrupted in a modular fashion.
Collectively, these observations suggest that the domains to study for genomic alterations, resulting in tissue-specific misregulation of a developmental gene and a subsequent malformation, should be much broader than traditionally investigated.

S15.1
How much of *de novo* is meiotic?

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Owing to the size and inherent instability of the human genome, more than one mutation is estimated to arise per mitotic division, resulting in somatic mosaicism. During the ~10^{16} mitotic cell divisions required to generate an adult, mosaic mutations can go unnoticed, underlie genetic disease or contribute to normal human variation, and may be transmitted to the next generation as constitutional variants. Early somatic mosaic mutations in the precursor somatic cells that will eventually constitute the germline can cause unexpected intergenerational recurrences of genetic and genomic disorders with multiple affected children born to unaffected parents, contrary to Mendelian expectations. Using the sensitivity of individual-specific breakpoint PCR, we prospectively screened 100 families with children affected by genomic disorders due to rare deletion CNVs determined to be *de novo* by clinical analysis of parental DNA. We found that an under-recognized and significant fraction of apparently *de novo* CNVs are not meiotic in origin, but rather arise during early post-zygotic mitoses and this can be identified either in the affected patients or their healthy parents. Our probabilistic model of gametogenesis to consider parental mosaicism as a source of transmitted mutations predicts that despite the fact that maternally transmitted mutations are the minority of alleles, a greater proportion of somatically mosaic transmitting mothers are at increased risk of recurrence. I will review the influence of the developmental timing of mutations, the mechanisms by which mutations arise, methods for detecting mosaic variants, and the risk of mosaic mutations being passed on to the next generation.

S15.2
Selfish mosaicism: impact of somatic mutations occurring in the paternal germline

A. Goriely;
Oxford, United Kingdom.

As mutations are at the origin of all genetic variations, understanding the factors that influence the apparent rate at which de novo mutations occur is crucial to the study of genome diversity, evolution and diseases. Although it is well established that point mutations initially arise as random miscopying events, preferentially from the paternal germline, we have described a new mechanism which predicts that certain pathogenic mutations may hijack the way sperm production is controlled to their own advantage. In doing so, these 'selfish' mutations become progressively enriched in the testis as men age and are therefore associated with an increased risk of transmission to the next generation.
The concept of selfish spermatogonial selection was originally proposed to account for the unusual presentation of a group of rare Mendelian diseases, which we collectively called 'paternal age-effect disorders'. It relies on principles similar to oncogenesis to explain why some paternally-derived mutations, such as those causing Apert (FGFR2) and Costello (HRAS) syndromes or achondroplasia (FGFR3), occur spontaneously at levels up to 1000-fold higher than the genomic background rate. The evidence - gathered originally through direct quantification of these ultra-rare pathogenic mutations in human sperm - suggests that selfish mutations, although occurring rarely, confer a selective advantage to mutant spermatogonial stem cells, leading to their clonal expansion and progressive enrichment in sperm over time.
Our understanding of this process so far suggests that molecularly selfish selection relies on the activation of the growth factor receptor-RAS signalling pathway, which is a key regulator of stem cell homeostasis in the testis. As RAS is required in many different cellular contexts, we will discuss to which extend dysregulation of this pathway is likely to be relevant to the pathology of common disorders, including cancer predisposition and neurodevelopmental disorders, such as schizophrenia and autism - for which paternal age-effects have been described epidemiologically.

S15.3
Somatic mutations in monozygotic twins

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S16.1
The human Y chromosome in evolution and disease

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S16.2
Ancient pathogen genomics of re-emerging infectious diseases

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Genome wide data from ancient microbes may help to understand mechanisms of pathogen evolution and adaptation for emerging and re-emerging infectious disease causing agents. Using high throughput DNA sequencing in combination with targeted DNA enrichment protocols we have reconstructed medieval bacterial genomes of *Yersinia pestis*, *Mycobacterium leprae* and *Mycobacterium tuberculosis* from ancient skeletal remains. Phylogenetic analysis indicate that the ancient *Y.pestis* strain from the Black Death pandemic is ancestral to most extant strains and falls very close to the ancestral node of human infectious *Y. pestis* bacteria. Temporal estimates suggest that the Black Death of 1346 - 1351 was the main historical event responsible for the introduction and worldwide dissemination of currently circulating *Y. pestis* strains pathogenic to humans, and further indicates that contemporary *Y. pestis* epidemics have their origins in the medieval era. In contrast the medieval *M. leprae* strains fall within the current genetic diversity and are found on at least two main branches in the phylogenetic tree of leprosy bacteria. The reconstructed *M.tuberculosis* genomes from 1000 year old Peruvian genomes, however, cluster together with *M.tuberculosis* strains found in modern mammalian species, suggesting a zoonotic origin for tuberculosis in the pre-columbian New World, likely introduced into human populations by contact to sea mammals. Dating analysis reveal a most recent common ancestor of *Y.pestis, M.leprae* and all *M.tuberculosis* strains within the last 6000 years, suggesting that all three human pathogens may have a recent Neolithic origin.

S16.3
Evaluating human genetic (and epigenetic) adaption to pathogen pressures

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Different environmental, demographic and selective forces, together with cultural and social characteristics of human lifestyle, shape the patterns of variability of the human genome at the population level. In particular, infectious diseases have been a major cause of human mortality, so natural selection is expected to act strongly on host defence genes. This is particularly expected for innate immunity genes, as they represent the first line of host defence against pathogens. I will present different cases of how some

of these genes and the pathways they trigger have been targeted by natural selection, in its different forms and intensities, helping to delineate genes that are important for host defence, with respect to those exhibiting higher immunological redundancy. I will also discuss how population-specific genetic variation can profoundly impact immune-related molecular phenotypes, such mRNA and miRNA expression upon infection (response eQTLs), and how these studies increase our understanding of immunological mechanisms under genetic control that have been crucial for our past and present survival against infection. Finally, I will discuss how the differences in lifestyle and habitat of human populations, together with their distinct patterns of genetic diversity, affect the epigenetic landscape of the human genome. Specifically, our studies of populations of African rainforest hunter-gatherers and sedentary farmers show that methylation variation associated with recent changes in habitat mostly involves immune functions, whereas that associated with historical lifestyle primarily affects developmental processes. Furthermore, methylation variation - particularly that correlated with historical lifestyle - shows strong associations with nearby genetic variants that, moreover, are enriched in signals of natural selection. Our work increases our understanding of whether and how populations are able to respond/adapt to environmental changes, including those related to pathogen pressures, and provides a resource for assessing the role of (epi) genetic mechanisms in human adaptation over different time scales.

EDUCATIONAL SESSIONS

ES1.1
Applications of CRISPR-Cas9 for Genome Engineering

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ES1.2
CRISPR-Cas9: biological roles, mechanisms, evolution and applications

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The RNA-programmable CRISPR-Cas9 system has recently emerged as a transformative technology in biological sciences, allowing rapid and efficient targeted genome editing, chromosomal marking and gene regulation in a large variety of cells and organisms. In this system, the endonuclease Cas9 or catalytically inactive Cas9 variants are programmed with single guide RNAs (sgRNAs) to target site-specifically any DNA sequence of interest given the presence of a short sequence (Protospacer Adjacent Motif, PAM) juxtaposed to the complementary region between the sgRNA and target DNA. The system is efficient, versatile and easily programmable.
Originally, CRISPR-Cas is an RNA-mediated adaptive immune system that protects bacteria and archaea from invading mobile genetic elements (phages, plasmids). Short crRNA (CRISPR RNA) molecules containing unique genome-targeting spacers commonly guide Cas protein(s) to invading cognate nucleic acids to affect their maintenance. CRISPR-Cas has been classified into three main types and further subtypes. CRISPR-Cas9 originates from the type II CRISPR-Cas system that has evolved unique molecular mechanisms for maturation of crRNAs and targeting of invading DNA, which my laboratory has identified in the human pathogen *Streptococcus pyogenes*. During the step of crRNA biogenesis, a unique CRISPR-associated RNA, tracrRNA, base pairs with the repeats of precursor-crRNA to form anti-repeat-repeat dual-RNAs that are cleaved by RNase III in the presence of Cas9 (formerly Csn1), generating mature tracrRNA and intermediate forms of crRNAs. Following a second maturation event, the mature dual-tracrRNA-crRNAs guide the endonuclease Cas9 to cleave cognate target DNA and thereby affect the maintenance of invading genomes. We have shown that the endonuclease Cas9 can be programmed with sgRNAs mimicking the natural dual-tracrRNA-crRNAs to target site-specifically any DNA sequence of interest. I will discuss the biological roles of CRISPR-Cas9, the mechanisms involved, the evolution of type II CRISPR-Cas components in bacteria and the applications of CRISPR-Cas9 as a novel genome engineering technology.

ES2.1
Leveraging molecular networks to reveal pathways underlying complex diseases

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Genome-wide association studies (GWASs) have successfully identified thousands of genetic loci associated with a broad range of complex traits and diseases. However, translating these associations into a functional understanding of disease processes remains a difficult problem. One approach to address this challenge are network-based association strategies, which interpret genetic variants within the molecular circuits that sense and propagate them. The premise is that, for a given trait or disease, causal variants tend to perturb genes that cluster in relevant pathways or network modules. Based on this premise, methods identify dysregulated pathways and/or prioritize candidate genes using network connectivity.
In this educational session, I will: (1) review basic concepts as well as the latest developments in pathway and network-based GWAS analysis, (2) discuss different types of networks that are used by these methods and how they can be constructed, and (3) give a brief summary of our recent work. We inferred a unique compendium of ~400 cell type and tissue-specific enhancer-gene regulatory networks and demonstrated their value for integration with GWAS data across a broad range of traits and diseases. Our results suggest that cell type-specific regulatory circuits are key to understand the fine-scale mechanism of genes underlying complex diseases. Web resources: http://dream.broadinstitute.org, http://regulatorycircuits.org.

ES2.2
Gene co-expression networks
L. Serrano;
Barcelona, Spain.

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ES3.1
Inherited and acquired kidney cancers: opportunities for targeted therapeutic approaches
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Hereditary renal cell carcinoma (RCC) may account for 5% to 8% of kidney cancers. This approximation may be significantly underestimated. However, the presentation will be focused on those inherited syndromes with kidney cancers, which have been associated to germline mutations in possibly "actionable" genes. Several hereditary RCC syndromes have been characterized, including von Hippel-Lindau (VHL), hereditary papillary renal cell carcinoma (HPRC), Birt-Hogg-Dube´ (BHD), hereditary leiomyomatosis and RCC (HLRCC), succinate dehydrogenase kidney cancer (SDH-RCC), tuberous sclerosis complex (TSC) and Cowden syndrome and have been shown to be associated with germline mutations in VHL, MET, FLCN, FH, SDHB/C/D, TSC1/2 or PTEN respectively. Although these syndromes have similarities, they vary in histology, aggressiveness, penetrance, and associated clinical manifestations. Understanding the genetic basis of cancer of the kidney, and in particular of the inherited forms, has significant implications for diagnosis and management of this disease and has provided the foundation for the development of targeted therapeutics. VHL is the gene for clear cell kidney cancer. The VHL protein forms a complex that targets the hypoxia-inducible factors (HIFs) for degradation. Knowledge of this pathway resulted in the development of therapeutic approaches now approved for treatment of this disease. Clinical trials are ongoing with agents targeting the tyrosine kinase MET in type I papillary RCC. BHD is thought to be involved in energy and/or nutrient sensing through the AMPK and mTOR signaling pathways. A hereditary form of type 2 papillary renal carcinoma is caused by inactivation of a Krebs cycle enzyme (FH or SDH) due to mutation. Also in these cases HIF might have a pathogenic role, but also other metabolic and signaling alterations might be targeted for therapy.

ES3.2
From inherited breast/ovarian cancer to PARP inhibitors and beyond
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It is now 20 years since the BRCA1 and BRCA2 genes were localized and identified. We know a tremendous amount about how these genes work, and great strides have been made in applying this knowledge clinically, most notably in the development of novel therapeutic approaches to cancers arising in mutation carriers. In addition, detailed, large-scale epidemiological studies have resulted in accurate estimations of cancer risks, and modifier genes are likely to be useful in the future to help stratify mutation carriers into clinically-useful risk categories. Despite these advances, 85% of the familial risk, and 60% of strongly heritable breast cancer remains unexplained, and similarly, a significant fraction of the genetic causes of ovarian cancer remains unexplained.
In this presentation, I will discuss newer breast and/or ovarian cancer susceptibility genes such as PALB2, ATM, CHEK2 and newly identified candidate genes such as RECQL, and others. First, I will discuss the genes, their key functions and their contribution to breast/ovarian cancer risk. Then I will introduce gene panel testing for breast/ovarian cancer, and consider the candidacy of these genes in the setting of testing unaffected women in the medical genetics clinic. Following on from this, I will debate the pros and cons of population-based testing for BRCA1, BRCA2 and other related genes. Finally, I will review data that relates to the use of germ-line genetic information about breast/ovarian cancer genes in making therapeutic decisions.

ES4.1
Patient perspective's to rare diseases
Y. Le Cam;
Paris, France.

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ES4.2
European rare disease policies- what does it really mean for planning services?
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Rare diseases are chronically debilitating conditions, mostly inherited, that affect less than 5 in 10000 people. It is estimated that in the EU 5,000-8,000 rare diseases affect 27-36 million people (6-8% of the population).
The EU objectives in the field of rare diseases have been defined in order to assemble all the elements necessary for a global strategy to tackle rare diseases efficiently. The foremost aim is to improve patients' chances of obtaining the most appropriate and timely diagnosis, information and care on the assumption that this aim can be achieved more effectively through collaborative, European action than by the Member States individually.
Most EU policies do not differ significantly from what most Member States already have in place and these policies should not incur an extra burden for the Member States, particularly those with greater economic challenges and less-developed health systems.
In a nutshell, EU rare disease policies are aiming to make rare diseases more visible through the promotion of an appropriate coding system that will allow the correct and prompt identification of patients. Other key aspects include the development and implementation of National Plans to ensure equal access to prevention, diagnosis, treatment and rehabilitation for people with rare diseases. In general, the European rare diseases policies ensure that common guidelines are developed and shared everywhere in Europe for areas such as research, centres of expertise, development of orphan drugs etc.
The concept of European reference networks (ERNs) for rare diseases is one of the latest instruments provided by the EC to tackle rare diseases requiring specialised care. They should serve as research and knowledge networks, treating patients from other Member States and ensuring the availability of subsequent treatment facilities where necessary. This concept will certainly support the implementation of the "Cross-Border Healthcare Directive" and the equity of care for patients according to the state of the art; however, it will also be the measure that will likely inflict greater financial cost on Member States. To establish effective ERNs will require not only technical but also financial resources coupled with an EU-wide spirit of cooperation.

ES5.1
GeneConsult, Phenomizer, Face2gene with short presentations how they work and test cases to compare the tools
P. Robinson;
Berlin, Germany.

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ES5.2
Clinical Face Phenotype Space: Using standard facial imaging to aid diagnosis of genetic syndromes
M. Alvi[1], Q. Ferry[1], J. Steinberg[1], C. Webber[1], D. R. FitzPatrick[2], C. P. Ponting[1], A. Zisserman[1], **C. Nellaker**[1];
[1]University of Oxford, Oxford, United Kingdom, [2]MRC Human Genetics Unit, Edinburgh, United Kingdom.

Clinical dysmorphology is a key discipline within clinical genetics and requires an enormous breadth of experience to correctly classify and diagnose ultra-rare diseases. There has been a significant amount of research into developing objective quantification methods to leverage clinician expertise. I will give a brief introduction to imaging based computational phenotyping research, touching on the morphometrics, anthropometry, 3D imaging modalities, and syndrome classification.
Recent developments in computer vision research have begun to enable analyses based on ordinary photographs to be performed for the purpose of computational phenotyping. I will present Clinical Face Phenotype Space (CFPS), an algorithm to automatically detect faces in photographs, annotate locations of key anatomical parts and extract machine readable feature descriptions of the facial gestalt. The approach uses machine learning to create a multidimensional space shaped to account for spurious variations such as lighting, pose, occlusions, and image quality. The CFPS locates patients in the context of known syndromes, and thus can help generating disease hypotheses. This holds promise as an impartial means of narrowing the search space to suspected rare diseases, and could augment the prioritisation of testing in clinical investigations. Moreover, CFPS allows the clustering of patients by phenotype even when no known syndrome diagnosis exists, thereby aiding disease identification.

Finally, I will describe new approaches to learn the CFPS models and the increased accuracies of facial phenotype representations, and how models of phenotype variation can aid inferring causative genetic variants from clinical sequencing.

ES6.1
The 100,000 Genomes Project, Bringing Personalised Medicine Into Healthcare

M. Caulfield;
London, United Kingdom.

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ES6.2
My vision on genomic medicine

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Consumer genetic testing and big data is a powerful combination proven to have a significant impact on research. Large pools of genetic information - like that being collected in the UK, China and United States - can help find treatments for disease and fuel scientific discoveries. But it begins with people and their engagement. A people-powered research model is critical to advancing research.

ES7.1
Imprinting and long noncoding RNAs in health and disease

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Imprinted genes are expressed from a single parental allele and are the main reason why mammals require both a maternal and paternal contribution for normal development. Defects in imprinted gene expression or loss of a single allele of an imprinted gene are responsible for a number of human syndromes, including Prader-Willi Syndrome, Angelman Syndrome, Beckwith-Wiedemann Syndrome and Silver-Russell Syndrome. There are fewer than 200 imprinted genes that have been described thus far in mice. The imprinted expression and regulation of these genes is largely conserved between rodents and humans, which allows mechanistic studies of this most unusual form of gene expression using both animal models and human tissues and cell models. Most imprinted genes reside in clusters that are located throughout the mammalian genome. The clusters typically contain an imprinting control region (ICR), which harbors allele-specific DNA methylation and governs the imprinting of the entire domain. Deletion or inappropriate epigenetic modifications of the ICR causes deregulation of the entire cluster in cis. Although most imprinted clusters use long non-coding RNAs to regulate imprinted gene expression, a few are regulated by CTCF and allele-specific insulator function. One such cluster harbors the H19 and Igf2 imprinted genes, and is controlled by an ICR that contains multiple CTCF binding sites. Gain of maternal methylation and loss of paternal hypermethylation of the H19/IGF2 ICR are associated with the human growth disorders Beckwith-Wiedemann Syndrome and Silver-Russell Syndrome, respectively. Using gene targeting and genome editing, we have generated ES cells, iPS cell lines and mice to study the mechanisms of imprinting for these imprinted loci and to model the epigenetic mutations in human syndromes. We have also developed SNP-FISH to study the dynamics of allele-specific gene expression at the single cell level in cell lines and tissues exhibiting loss of imprinting.

ES7.2
Diagnosing imprinting-related disorders

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Genomic imprinting is a good example of epigenetic regulation of gene expression. Imprinted genes are characterised by expression from only one allele (of the pair) in a consistent parent of origin manner; often only in certain developmental windows and in some tissues of the body. The pattern is set by targeted methylation within the male or female germ line that resists the post fertilisation waves of demethylation of the zygote. Imprinted genes play an important role in fetal growth and development. Their carefully regulated expression is important for normal cellular metabolism and human behaviour.
Imprinting Disorders.
A genetic or epigenetic mutation that impacts imprinting expression can result in one of eight clinically recognisable disorders:-
1. Silver Russell syndrome
chrm 7, 11p15 (Growth restriction, asymmetry)
2. Transient Neonatal Diabetes Mellitus (TNDM)
6q24 (Transient neonatal diabetes, intrauterine growth retardation, diabetes)
3. Beckwith-Wiedemann syndrome (BWS)
11p15 (Overgrowth, omphalocoele, hypoglycaemia, tumour risk)
4. Wang-Kagami-Ogata syndrome (WKOS)
14q32 (mild overgrowth, mental retardation)
5. Temple syndrome (TS)
14q32 (Growth retardation, early puberty, obesity)
6. Angelman syndrome (AS)
15q11q13 (Mental retardation, ataxia)
7. Prader-Willi Syndrome (PWS)
15q11q13 (Growth retardation, mental retardation, obesity)
8. Pseudohypoparathyroidism Ib (PHP1b)
20q13.2 (Overgrowth, parathyroid hormone resistance)
The major overlapping features in most of these conditions (except Angelman) are disordered fetal growth, either excessive or restricted growth, neurodevelopmental delay and disordered metabolism. Diagnosis can be difficiult as many of the features are non-specific.
Most patients with IDs either have uniparental disomy (inheritance of both chromosome homologues from one parent with no contribution from the other), copy number variation involving imprinted loci, imprinted gene mutation or an epigenetic mutation.
To be discussed:
-examples of each phenotype together with the clinical challenges for diagnosis
-genetic counselling and risks to sibs and offspring dependent on the underlying epigenetic or genetic mutation

ES8.1
Wishes for the end of life in Huntington's Disease

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Introduction: In the Netherlands euthanasia or physician assisted suicide (PAS) is legal since 2002 under strict conditions. Euthanasia or PAS is exempt from prosecution when the euthanasia or PAS is performed by a physician after a direct request from a patient or based on an advance directive. Furthermore the physician must be convinced that the patient suffers unbearable and without prospect or relief.
Huntington's Disease (HD) is an autosomal dominant progressive neurodegenerative disease, characterized by chorea and hypokinesia, psychiatric symptoms and progressive cognitive decline leading to dementia. In The Netherlands approximately 1,700 patients have HD and another 6,000-8,000 persons are at risk. Primary cause of death is pneumonia, second cause is suicide. Each year approximately 7 requests for euthanasia from a HD patient are granted. We have observed an increase in conversations about wishes for the end of life between physicians and patients, but this was never investigated.
Objective: to investigate presence and content of wishes for the end of life amongst HD patients, and the relationship with demographic or disease specific characteristics.
Methods: First, 14 patients in different stages of the disease and 15 physicians were interviewed. Based on the qualitative research a custom made questionnaire was developed and sent to 242 Dutch patients registered in the Leiden HD Registry Database. Information on demographic variables and clinical characteristics such as TFC, MMSE, UHDRS-M was collected.
Results: The interviews showed that most patients had strong ideas about things they do not want for the end of their life because of their experiences with family members. Knowledge about how to effectuate the wishes (for example the role of the physician or family and the value of the advance directive) was in some of them inadequate or lacking. In general, conversations about wishes for the end of life were not initiated by physicians. Physicians reported problems how to deal with an advance directive especially in light of cognitive decline, psychiatric symptoms and signs and 'response shift'.
The questionnaire was returned by 134 patients (55.4%) of whom 101 (75%) reported to have some kind of thoughts for the end of life. These thoughts concerned care (11%) and ; thoughts about euthanasia or PAS (64%). The presence of thoughts about the end of life was related to being familiar with HD but not to any other socio-demographic or clinical variable.
Conclusion: Thoughts about and wishes for the end of life are widely present amongst patients with HD and known gene carriers and these thoughts or wishes concern euthanasia or PAS in a majority of the respondents. Kno-

wing if end-of-life wishes are indeed increasingly present and knowing the content of these wishes could be helpful for physicians. It
could help treatment and guidance of HD patients, especially in light of the absence of any effective treatment. Based on this study it seems impossible to determine which patients will have thoughts or wishes for the end of life and which patients do not. Therefore these questions should be addressed in every patient with HD.

ES8.2
End of life decision making in neonates

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In the developed world, gone are the days were infants simply died. Now, almost every death is preceded by some exposure to medical care, where diagnoses and prognoses are rendered and decisions are made. The same is true of infants who survive life-threatening illness: they have often been through a long gauntlet of medical interventions. As a result, keeping track of all the care and decisions that preceded the current moment in time is essential to understand how the population of infants being observed came into existence, and to what we can validly infer from comparisons of groups of infants. And as our ability to intervene in the lives and deaths of these patients increases, and the decisions of what to do or not do hinge not only on data but also diverse human values, the importance of understanding and monitoring how infants die increases in equal measure. In this presentation, the most common decisions about life-sustaining interventions in sick newborn are being discussed. Special attention is given to recent developments in practices such as neonatal euthanasia (in some countries) and withdrawal of artificial fluid and nutrition that have may rendered boundaries between withholding and withdrawing life-saving treatments more complex.

ES9.1
Functional prediction of DNA sequence changes

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No abstract received as per date of production. Check http://www.eshg.org/abstracts2015.0.html for possible updates.

ES9.2
Protein structures to advance therapeutic discoveries

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No abstract received as per date of production. Check http://www.eshg.org/abstracts2015.0.html for possible updates.

CONCURRENT SESSIONS

C01.1
Implementation of a non-invasive prenatal testing (NIPT) for aneuploidy service in an NHS diagnostic laboratory

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Non-invasive prenatal testing (NIPT) for aneuploidies is widely available through commercial providers, but implementation into public sector maternity care requires detailed evaluation because of the likely changes in care pathway, educational requirements and potential economic impact.
Here we report the results of a study where a massively parallel sequencing approach to aneuploidy was developed in our NHS Regional Genetics Laboratory, along with health professional and patient educational materials before introducing it into the DSS pathway in eight UK maternity units. Before the study we estimated that if NIPT cost ~£250 per case introduction as a contingent test at a cutoff of 1:1000 would increase the DS detection, whilst decreasing invasive testing with slight overall increase in cost of the DSS and diagnostic pathway.
Women with a DSS risk ≥1:1000 were offered NIPT for trisomies 21, 18, and 13. The study, in a population of ~40,000 women, resulted in over 2500 undergoing NIPT with results reported within 10 days, demonstrated the feasibility of introducing NIPT into the DSS pathway in the NHS. Women were very positive about NIPT, invasive testing rates fell significantly and the detection of aneuploidy increased subsequent to the detection of aneuploid cases in the intermediate (1:150-1:1000) risk group. The higher than expected uptake of testing, some for information only, indicates that for introduction of NIPT to be cost neutral, a lower risk cut off will be required. These data are informing a report to the UK National Screening Committee who are responsible for the UK DSS policy.

C01.2
TRIDENT: or monitored NIPT implementation in the Netherlands

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In many countries, Non Invasive Prenatal testing (NIPT) has been introduced commercially, without governmental guidance. In the Netherlands the Population Screening Act regulates the introduction of screening programs for untreatable diseases such as Down syndrome. The Dutch NIPT consortium, consisting of all relevant stakeholders, obtained a license for 2 years for a nationwide NIPT implementation study called TRIDENT (Trial by Dutch laboratories for Evaluation
of Non-Invasive Prenatal Testing). The study started on April 1st 2014. Inclusion criteria are an increased risk (>1:200) for trisomy (T) 21, 18 or 13 based on the first trimester combined test, or because of medical history. After nine months of study, 2439 pregnant women have been tested and 2425 (99,4%) reports issued. We found 70 cases of T21 (2,9%), 8 cases of T18 (0,3%) and 10 cases of T13 (0,4%). Follow-up was completed for 78 cases of which 72 were confirmed, 6 were false positives, presumably due to confined placental mosaicism. Median turnaround time was 13 working days. Data on pregnancy outcomes are currently being collected. An amendment to TRIDENT to offer NIPT to all pregnant women as a first tier test is momentarily pending.
The Netherlands are the first country where NIPT is incorporated into a go-

vernmentally supported and health care funded prenatal Down syndrome screening program. The incorporation of the test in a university hospital laboratory and clinical service guarantees appropriate counselling and allows for proper follow up including thorough exploration of biological causes of false positive and false negative findings including detailed placental examination.

C01.3
Non-invasive prenatal diagnosis; expansion from de novo to autosomal recessive disorders using congenital adrenal hyperplasia as an example

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Introduction
Our public sector accredited laboratory offers non-invasive prenatal diagnosis (NIPD) but currently this is restricted to paternal exclusion and de novo mutations. Here we review our experience of NIPD and introduce congenital adrenal hyperplasia (CAH) as a model for NIPD of autosomal recessive (AR) disease.
Materials and methods
An audit of cases was carried out from our internal database for 2014.
Agilent Sureselect Custom enrichment assay, targeted at heterozygous SNPs around CYP21A2 was designed. CffDNA was prepared using Kappa Hyper Prep Kit. Libraries were sequenced on the Illumina MiSeq and variants called using BWA and Varscan. Parental and proband samples were used to construct parental haplotype. Fetal inheritance was determined using the constructed haplotypes.
Results
In 2014 203/337 (60%) molecular prenatal tests in our laboratory were non-invasive. NIPD for fetal sex determination accounted for 73%, 14% were for achondroplasia, 13% thanatophoric dysplasia and around 0.5% for other monogenic disorders. Of those giving a definitive diagnosis in a monogenic condition 63/197 (32%) were NIPD.
CAH analysis of two families to date has correctly identified fetally inherited alleles, consistent with either corresponding CVS sample or follow-up data from the pregnancy.
Conclusions
Non-invasive referrals exceed invasive in our prenatal service. NIPD in our laboratory is considered diagnostic and confirmatory invasive testing is not recommended. We are validating non-invasive assays for other recessive conditions including cystic fibrosis, sickle cell and beta-thalassemia.
Supported by International Fund Congenital Adrenal Hyperplasia and European Society for Paediatric Oncology, NIHR and GOSH-UCL BRC and GOSHCC

C01.4
Non-invasive prenatal diagnosis (NIPD) of Duchenne and Becker muscular dystrophies (DMD/BMD) by relative haplotype dosage

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As part of the NIPSIGEN project (Non-Invasive Prenatal diagnosis for Single Gene disorders), we are developing and validating a clinical test for NIPD of DMD/BMD in at-risk pregnancies. Through highly targeted enrichment (200 Kb across a 2.4 Mb region on ChrX) and massively parallel sequencing (Illumina MiSeq) of cffDNA followed by relative haplotype dosage (RHDO) analysis, we were able to determine fetal inheritance of the dystrophin gene in six male pregnancies: four healthy pregnant donors and two pregnant DMD carriers all undergoing invasive prenatal testing. Fetal genomic DNA from CVS of healthy donors was used to identify the haplotype of interest on ChrX. For the DMD pregnancies, the affected haplotype was identified from genomic DNA from an affected sibling. Using RHDO analysis, the allelic imbalance in 300-400 heterozygous SNPs was used to determine over /under-representation of the reference haplotype in statistically independent haplotype blocks. For all healthy pregnancies, over-representation of the expected fetal haplotype was observed for all haplotype blocks. Both DMD pregnancies were correctly diagnosed and a recombination event was detected. The fetal portion of cfDNA inputted into the RHDO analysis was calculated using the same allelic imbalance. This new assay for NIPD of DMD/BMD has shown great promise in the initial stages of validation with high sensitivity and specificity on samples tested so far. It is highly affordable (2-3 patients per sequencing run) and capable of detecting recombination events within the DMD gene by splitting the RHDO analysis into multiple statistically independent haplotype blocks across the targeted region.
Funded by: Health Innovation Challenge Fund (HICF) (DoH, Welcome Trust).

C01.5
Incidental findings of genome wide non-invasive fetal aneuploidy detection (NIPT): presymptomatic identification of maternal cancers

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Non-invasive prenatal testing (NIPT) for fetal aneuploidy detection is increasingly being offered in the clinical setting following studies demonstrating high sensitivities and specificities for trisomies 21, 18 and 13 detection. However, a baseline false positive and false negative rate remains. We introduced an analysis pipeline which addresses some of the technical and biologically-derived causes of error. Importantly, it differentiates high z-scores due to fetal trisomies from those due to local maternal CNVs causing false positives. Following routine clinical analysis of over 5000 prospective pregnancies, several other genomic imbalances were found in addition to detection of the common autosomal aneuploidies. These findings include (i) other (segmental) aneuploidies (0,3% of cases), 4 of which could be confirmed to be fetal (1 mosaic trisomy 16 and 1 mosaic trisomy 15 which contained a uniparental disomy cell line, 2 partial trisomies 18 and 1 terminal 5p deletion) and (ii) maternal imbalances. Importantly, three aberrant genome representation profiles were observed that could not be attributed to the maternal nor the fetal genomic constitution. Whole body diffusion-weighted magnetic resonance imaging and subsequent pathologic and genetic investigation uncovered the presence of respectively an ovarian carcinoma, a follicular lymphoma and a nodular sclerosis classical Hodgkin lymphoma. The copy number variations in those tumors were concordant with the NIPT plasma profile. NIPT thus enables the accurate presymptomatic detection of maternal tumors. These incidental findings are an unsuspected added benefit of NIPT.

C01.6
Non-invasive prenatal testing for aneuploidy and beyond: challenges of responsible innovation in prenatal screening - an ESHG/ASHG position statement

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Several professional societies have issued position statements on noninvasive testing (NIPT) in prenatal screening for common autosomal aneuploidies. Ethical aspects have not been a main focus of those statements. This ESHG/ASHG document fills this lacuna. Emerging scenarios for introducing NIPT into prenatal screening should not just be regarded as a matter of screening technology and health economics; the question is also how the trade-offs involved enable or impede meaningful reproductive choices and how they affect the balance of benefits and burdens for pregnant women and their partners. With improving screening technologies and decreasing costs of sequencing and analysis, it will become technically possible to expand the scope of prenatal screening beyond common autosomal aneuploidies. This should be limited to serious congenital and childhood disorders, and only following sound validation studies and a comprehensive evaluation of all relevant aspects. In countries where prenatal screening for fetal abnormalities is offered as a public health programme, governments and public health authorities should adopt a more active role to ensure the responsible innovation of prenatal screening on the basis of ethical principles. This requires guarantees of the quality of the screening process as a whole (including non-laboratory aspects such as information and counseling), education of professionals, systematic evaluation in the light of the aim of the practice, accountability to all stakeholders and promotion of equity of access. This document is the result of a unique collaboration between the ESHG Public and Professional Policy Committee, and the ASHG Social Issues Committee. The final version was approved by the Boards of both Societies in December 2014.

C02.1

Single cell analysis "simplification" dramatically increases complexity: considerations in technique, quality control, analysis, and possibilities for translation to the clinic

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Introduction: New technology platforms intended for single cell analysis (SCA) are bringing us to a new frontier in science and studies that were only dreamt are very possibly within reach of researchers studying everything from population dynamics, cell signaling, to cancer/metastases mutation detection, and eventual move of SCA into diagnostics/personalized medicine. We report on the challenges involved in developing SCA for RNA NGS: workflow, experimental design, techniques, quality control, analysis, and interpretation.
Materials and Methods: Utilizing Fluidigm's C1™ system, we captured 4 cell populations. cDNA was QCd with capillary electrophoresis. 96 single cells were prepped for Illumina sequencing using a modified Nextera XT kit. Samples were sequenced on the HiSeq2500 Rapid (PE 100 base reads).
Results: Integrated workflow between flow sorting and C1 capture required planning and a close relationship with the flow facility. Although cDNA, library preparation, and the sequencing run QC measurements passed, we had 14% failure (13/96 libraries). Data analysis (approximately 3 million reads/cell) demonstrates that accumulation of unique RNA species across multiple single cells yields coverage estimates for genomic expression, but that cell types can be differentiated.
Conclusions: SCA has great potential, however, variance in expression of individual genes requires detailed consideration, and cluster analysis of single cells from multiple compartments should take mean-variance relationships of individual genes into account. Analysis and experimental design require a mind shift from the averaging method to which we have been chained and there remains an inordinate amount of work to be done before single cell clinical diagnostics is possible.

C02.2

Large-scale genotyping of polymorphic inversions in the human genome

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Inversions are known to be associated to phenotypic and functional differences between individuals. Unfortunately, their study has lagged behind due to the technical difficulties of their analysis, especially when they are flanked by highly identical inverted repeats (IRs). Here, we present a new method called inverse MLPA (iMLPA, patent pending) to genotype simultaneously multiple inversions mediated by IRs in hundreds of individuals, which is based on a combination of inverse PCR and probe hybridization. In particular, current developed assays are able to genotype more than 30 inversions using only 25 ng of DNA per inversion, although it should be easy to include additional inversions as they are identified. To test the performance of the technique, we have genotyped 24 of these inversions in 550 individuals of seven diverse human populations from the 1000 Genomes Project with 98.5% genotyping success rate. In addition, by comparing with the results obtained by PCR for a subset of the samples, we have shown that iMLPA is highly accurate and most errors accumulate in specific inversions affected by restriction-site polymorphisms. Finally, we have established the population distribution and evolutionary history of the inversions, their functional effects on genes, and we have observed that most inversions with IRs are recurrent and are not linked to SNPs. Therefore, having a high-throughput technique to genotype these inversions is crucial to shed light on their association with complex phenotypes and disease susceptibilities, and could contribute to unravel the hidden heritability of the human genome.
Support: European Research Council (ERC) Starting Grant (INVFEST).

C02.3

Large-scale single-molecule sequencing of tandem repeats on the human X chromosome

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Tandem repeats are short DNA sequences that are repeated head-to-tail with a propensity to be variable. They constitute a significant proportion of the human genome, also occurring within coding and regulatory regions. Variation in these repeats can alter the function and/or expression of genes allowing organisms to swiftly adapt to novel environments. Importantly, some repeat expansions have also been linked to certain diseases. Unfortunately, due to the nature of short read sequencing technologies, tandem repeats are not analyzed during whole genome or exome sequencing studies. We developed a novel capture assay for large-scale genotyping of tandem repeats (Duitama J., Zablotskaya A. et al., Nucl. Acids Research, 2014) and extended the assay for the identification of X linked disease-related repeats using long read (averaging 12 kb) PacBio RS II technology. For 837 (83% of all) potentially functional repeats, unique capture baits were designed, as well as for 1000 intronic and intergenic repeats. Of these, a full tandem repeat length sequence was obtained for 84-89% of the targets in male DNA samples. We are currently implementing this assay to screen for potentially causal variation underlying X-linked disorders that are not explained following array and exome sequencing.
Grant references: Fonds voor Wetenschappelijk Onderzoek (FWO)-Vlaanderen [G.0795.11 to K.J.V., J.R.V., G.F.]; Marguérite-Marie Delacroix [GV/B-155 to A.Z., G.F].

C02.4

The value of long-read single molecule sequencing in diagnostics

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Sanger sequencing is THE standard to scan disease-genes for deleterious variants. Although next generation sequencing is gradually taken over it will not resolve all issues. A major limitation of both methods is their short read length (~1,000 nucleotides), making it impossible to study certain rearrangements and/or resolve regions with repetitive sequences. We have applied long-read single molecule sequencing (Pacific Biosciences) to overcome this limitation and tackle some of the remaining diagnostic problems.
For targeted sequencing we apply a two-step procedure: target amplification with gene-specific M13-tailed primers followed by barcoding using reusable M13-tailed barcode primers. Barcoded samples are then pooled and processed for smart-bell sequencing. Sequencing runs yield ~60,000 sequences with ~15kb read lengths.
Using this approach we have targeted several loci of interest. The CYP2D6 gene is amplified and sequenced as a 6.6kb fragment. Although homologous sequences may co-amplify (e.g. the CYP2D7 gene) the full length sequences simply their discrimination and recognition of both CYP2D6 alleles. The latter is important to accurately predict the drug dose to use. In PKD1 long-reads allow us to start in unique 3' sequences and read far into highly repetitive 5' sequences. In PMS2 they help to discriminate the active gene from several PMS2 pseudogenes. When genomic structure complicates amplification or compromises analysis, we use RNA to synthesize full-length cDNA and perform long-read sequencing. Finally, we use long-read single molecule sequencing to resolve individual alleles from repeat expansion disorders and to analyse complex disease-associated repeat structures (D4Z4 in FSHD). We conclude PacBio-sequencing is a powerful diagnostic tool facilitating analysis of complex genomic loci.

C02.5

Comparison of exome and genome sequencing technologies for the complete capture of protein coding regions

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For next-generation sequencing technologies sufficient base-pair coverage is the foremost requirement for the reliable detection of genomic variants. We investigated whether whole genome sequencing (WGS) platforms offer superior coverage of coding regions compared to whole exome sequencing (WES) platforms, and compared single-base coverage for a large set of different exome and genome samples (24 Agilent V4 (at 78x and 160x coverage), 12 Agilent V5 (100x), 12 NimbleGen V3 (95x), 24 Complete Genomics (44x and 87x), 11 Illumina HiSeq (28x), 12 Illumina X-Ten (40x)).

We find that WES platforms have improved considerably in the last years, but at comparable sequencing depth, WGS outperforms WES in terms of covered coding regions. At higher sequencing depth (95x-160x) WES successfully captures 95% of the coding regions with a minimal coverage of 20x, compared to 98% for WGS at 87 fold coverage. A comparison to published gene panel studies shows that these perform similar to WES and WGS in terms of coverage. Three different assessments of sequence coverage bias showed consistent biases for WES but not for WGS. We found no clear differences for the technologies concerning their ability to achieve complete coverage of 2,759 clinically relevant genes.
We show that WES performs comparable to WGS in terms of covered bases if sequenced at 2-3 times higher coverage. This does, however, go at the cost of substantially more sequencing biases in WES approaches, which may impact applications such as the identification of copy-number variants and somatic variation. Our findings will guide laboratories to make an informed decision on which sequencing platform and coverage to choose.

C02.6
A significant proportion of de novo point mutations arise post-zygotically

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De novo mutations are recognized both as an important source of human genetic variation and as a prominent cause of sporadic disease. Mutations identified as de novo are generally assumed to have occurred during gametogenesis and, consequently, be present as germline events in an individual. However, Sanger sequencing does not provide the sensitivity to reliably distinguish somatic from germline mutations. Therefore, the proportion of de novo mutations occurring somatically rather than in the germline remains unknown. To determine the contribution of post-zygotic events to de novo mutations, we analysed a set of de novo mutations in 50 parent-offspring trios using three sequencing techniques. We found that 8 out of 107 presumed germline de novo mutations (7.5%) were in fact present as mosaic mutations in the blood of the offspring and were therefore likely to have occurred post-zygotically. Furthermore, genome-wide analysis of de novo variants led to the identification of 5 de novo mutations in the offspring which were also detectable in the blood of one of the parents. This implies parental mosaicism as the origin of these de novo mutations in 5 out of 50 trios. Remarkably, none of the parental low-level mosaicisms detected by Whole Genome Sequencing and validated by amplicon-based deep sequencing, were identified by Sanger sequencing. Our results show that a significant proportion of de novo mutations presumed to be germline in fact occurred either post-zygotically in the offspring or were inherited from low level mosaicism in one of the parents.

C03.1
De novo and familial *DDX3X* mutations are associated with X-linked intellectual disability and a diverse phenotypic spectrum

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Next generation sequencing studies have led to the identification of a large number of novel autosomal intellectual disability (ID) genes. However, the interpretation of disease causality of genetic variants on the X chromosome has remained behind and up to now has been mostly dependent on segregation analysis in families. We identified *de novo* frameshift, missense, nonsense and splice site mutations in *DDX3X* in 15 females within cohorts of sporadic ID patients. These females showed mild to severe ID and various other features including cortical dysplasia, hypotonia, movement disorders, behaviour problems and epilepsy. In seeming contrast with these observations, we additionally identified three families with variants in *DDX3X* suggestive for a X-linked recessive inheritance pattern, in which males had mild to severe ID while carrier females were unaffected. Intriguingly, *in silico* prediction and protein modelling programs could not discriminate between the *de novo* variants in affected females and the variants associated with X-linked recessive inheritance. The gene *DDX3X* encodes a multifunctional RNA helicase and is particularly intolerant to genomic variation. To further explore the pathogenic mechanisms that might underlie the differences in disease transmission and phenotypic outcomes, we have employed a combination of in vitro and in vivo assays grounded on the known roles of DDX3X on the regulation of β-catenin signaling. Preliminary studies indicate a dosage-dependent effect of DDX3X on Wnt signaling that is altered by the introduction of the majority of the missense changes found in our cohort.

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C03.2
De novo and recurrent PPP2R5D and PPP2R1A missense mutations cause protein phosphatase 2A dysfunction and intellectual disability

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For the first time, inherited dysregulation of protein serine/threonine dephosphorylation is found to cause genetic disease. De novo missense mutations in two different subunits of protein phosphatase 2A (PP2A) were identified in 15 individuals with mild to severe ID, long-lasting hypotonia, epileptic susceptibility, frontal bossing, mild hypertelorism and downslanting palpebral fissures.
The PP2A holoenzymes comprise catalytic (C), scaffolding (A) and regulatory (B) subunits that determine subcellular anchoring, substrate specificity and physiological function. Nine patients had mutations (E198K, E200K, P201R or W207R) that brought a basic charge into a highly conserved acidic loop of the PPP2R5D-encoded regulatory B56δ subunit, including six individuals who had the same de novo E198K mutation. Five others had de novo mutations (P179L, R182W and R258H, all also cancer-associated) in the PPP2R1A-encoded scaffolding Aα subunit. Large ventricles causing macrocephaly and suspicion of hydrocephalus were features in some cases, and all Aα cases had partial or complete corpus callosum agenesis.
Functional studies showed that the mutant A and B subunits were stable and uncoupled from the C subunit: Mutant B56δ had deficient A and C subunit binding, while mutant Aα had deficient binding of C, but not of B56δ. This suggested that mutant B56δ or mutant Aα-B56δ complexes could hinder C subunit access to B56δ-anchored PP2A substrates. Such a dominant-negative effect was supported by our finding of hyperphosphorylation of B56δ-regulated substrates upon mutant subunit overexpression. This was also in line with clinical observation indicating a correlation between the degrees of ID and biochemical disturbance.

C03.3
Mutations in genes encoding components of protein phosphatase 2A (PP2A) cause human overgrowth and intellectual disability

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Overgrowth syndromes comprise a group of heterogeneous disorders characterised by excessive growth parameters, often in association with intellectual disability. To identify new causes of human overgrowth, we have been

undertaking trio-based exome sequencing studies in overgrowth patients and their unaffected parents. Prioritisation of functionally relevant genes with multiple unique de novo mutations revealed four mutations in three related genes encoding components of the protein phosphatase 2A (PP2A), a key cellular serine-threonine phosphatase. All four mutations clustered within a highly conserved functional domain of 27 nucleotides shared by all three genes. Analysis of exome sequencing data from a follow-up cohort of overgrowth probands identified a further mutation in the cluster region, bringing the total number of patients with PP2A-related mutations to five. Mutation carriers shared some similar phenotypic features including increased height, increased head circumference and intellectual disability. We mapped the mutations onto the crystal structure of the PP2A holoenzyme complex to predict their molecular and functional consequences. These studies suggest that the mutations may affect substrate binding, thus perturbing the ability of PP2A to dephosphorylate certain protein substrates. PP2A is a major negative regulator of V-akt Murine Thyoma Viral Oncogene Homolog (AKT). Thus, our data further expands the list of genes encoding components of the PI3K/AKT signalling cascade that are disrupted in human overgrowth conditions.

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C03.4

De novo mutations in BCL11A cause developmental delay: additional implications of the BAF SWI/SNF complex in intellectual disability and autism

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Mutations in chromatin remodelling and histone modification genes are increasingly recognized as a major cause of intellectual disability (ID) and other frequently overlapping neurodevelopmental disorders such as autism. BCL11A has recently been shown to be a stable subunit of the BAF SWI/SNF complex in mammals. This evolutionary conserved complex is involved in transcriptional regulation through ATP-dependent modification of chromatin structure.

The Deciphering Developmental Disorders Study (DDD) has identified de novo mutations in *BCL11A* (previously linked to autism spectrum disorder) as a cause of non-syndromic intellectual disability. We present the clinical features of these patients, as well as model organism studies that support the role of *Bcl11a* in neurodevelopmental phenotypes.

In mouse embryonic development, *Bcl11a* is expressed in the forebrain and derivatives of the first and second pharyngeal arches. It maintains high expression in the central nervous system and craniofacial mesenchyme throughout postnatal stages. The study of the haploinsufficient mouse model of *Bcl11a* shows abnormal brain development, with overall decreased brain size, particularly in regions of the limbic system. Behavioural phenotyping has identified impaired social behaviour in the haploinsufficient mice compared to littermate controls.

Previously known for its role in lymphoid malignancies, *BCL11A* now joins the increasing number of genes involved in neurodevelopment through a putative role in chromatin remodelling.

C03.5

De novo loss-of-function mutations in WAC in the 10p12p11 critical region cause intellectual disability

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Background – Trio-based exome sequencing in 100 patients with intellectual disability (ID) previously identified a *de novo* mutation in *WAC*, encoding a protein regulating transcription-coupled histone H2B ubiquitination. The *WAC* gene is located on chromosome 10p11.23 and has previously been implicated in a microdeletion syndrome also containing *BAMBI*. Here, we used different strategies to identify additional patients with mutations in *WAC* to establish the role of *WAC* in ID.

Methods - We collected *de novo* mutations affecting *WAC* through routine diagnostic procedures including genomic microarray analysis and/or exome sequencing, and supplemented these with mutations reported in (inter) national databases. Moreover, we performed targeted resequencing of *WAC* in 2,326 patients with unexplained ID. For patients with mutations, we performed a detailed phenotypic comparison to the previously described phenotype for 10p12p11 microdeletion syndrome.

Results – We identified four additional *de novo* loss-of-function mutations in *WAC*, including three point mutations and one partial deletion. Clinical re-evaluation of our total cohort of five patients revealed phenotypic overlap for mild ID, hypotonia, behavioral problems and distinct overlapping facial dysmorphism, including a square shape of the face, deep set eyes, Kabuki-like long palpebral fissures, broad mouth and broad chin. Notably, these clinical features are comparable to the main features observed in 10p12p11 microdeletion syndrome.

Conclusions –We defined a clinically recognizable ID syndrome, caused by *de novo* loss-of-function mutations in *WAC*, characterized by ID, hypotonia and distinctive facial features. In addition, our data suggest that haploinsufficiency of *WAC* contributes to the phenotypic appearance of patients with the 10p12p11 microdeletion syndrome.

C03.6

A novel syndrome of learning disability and obesity caused by 6q16 deletions encompassing the essential neurogenesis factor POU3F2 (Brn2) helps to delineate the neuro-endocrine pathway for body-mass control

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Studies of genetic causes of intellectual disability and identification of monogenic causes of obesity in humans have made immense contributions towards the understanding of the brain and control of body mass. Here we describe a novel disorder of global developmental delay, intellectual disability and susceptibility to obesity caused by 6q16 deletions encompassing POU3F2 (Brn2 or N-Oct3 or OCT7) in nine patients from five families. The developmental delay and intellectual disability varied from mild to severe in our cohort. The body mass index (BMI) of all, but one patient, was above the 99th centile and in the obese category. BMI of one girl in our study was on the 91st centile putting her in overweight category.

POU3F2 upregulates proneuronal genes, is required for production, migration and positioning of neocortical neurons, is an essential factor needed to generate induced neuronal cells in vitro and is required for hypothalamic development and function. Using zebrafish as model organism we show that pou3f2 lies downstream of sim1 in the leptin>melanocortin>Sim1 pathway and controls oxytocin expression in the hypothalamic neuroendocrine preoptic area. Our zebrafish work, previous work on mouse models and the human phenotypes demonstrate that the molecular pathway linking the genes in the central molecular pathway that regulates body mass is conserved across multiple species. This is further supported by our analysis of expression pattern of POU3F2 and related genes in human brain. This work helps to delineate the neuro-endocrine pathway for energy balance/food intake and its role in monogenic obesity.

C04.1

Mosaic loss of chromosome Y (LOY) in peripheral blood is associated with smoking, shorter survival and increased risk of cancer

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Smoking is a major preventable environmental risk factor related to human health. Smoking killed about 100 million people during the 20th century and is projected to kill one billion people during this century, assuming that the current frequency of smoking is retained. Lung cancer is the prime cause of cancer-associated death in relation to smoking. However, it is less well appreciated that smoking also causes tumors outside the respiratory tract, which are predominant in men, and cumulatively roughly as common as lung cancer. Moreover, it is known that males have a higher incidence and mortality from most sex-unspecific cancers, disregarding smoking status, and this fact is largely unexplained by known risk factors. We have shown that a male specific risk factor, acquired mosaic loss of chromosome Y (LOY) in non-cancerous blood cells, is associated with an increased risk of non-hematological tumors among aging males (Forsberg et al. 2014 Nature Genetics, PMID: 24777449). We have also recently shown that smoking is associated with LOY in blood cells in three independent cohorts (TwinGene: OR=4.3, 95% CI =2.8-6.7; ULSAM: OR=2.4, 95% CI=1.6-3.6; and PIVUS: OR=3.5, 95% CI=1.4-8.4) encompassing in total 6014 men. Our data also support a transient and dose-dependent mutagenic effect from smoking on LOY-status (Dumanski et al. 2015 Science, PMID: 25477213). Thus, smoking may induce LOY, linking the most common acquired human mutation with a severe preventable risk factor. Our results could explain the observed sex differences and why smoking seems a greater risk factor for cancer in men than women.

C04.2
SNP-SNP interaction analysis of NF-kB signaling pathway on breast cancer survival

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Aberrant regulation of the NF-κB pathway has been shown in breast cancer, however, the impact of the genetic variation in the pathway on patient prognosis has been little studied. Possibly, for a complex disease such as breast cancer a single SNP is not independently critical in the biological function underlying the initiation or progression of the disease but multiple loci might jointly exert a greater impact. We used an extensive data set of the Breast Cancer Association Consortium (BCAC) with 30,431 cases to investigate the NF-κB activating pathway for association between 917 germline SNPs in 75 genes and breast cancer survival. Assessing two-way SNP-SNP interaction survival analyses, we found two pairs of genetic variations with interactive effect on breast cancer survival, i.e. rs5996080 and rs7973914 (HRinteraction 6.98, 95% CI 3.3-14.4, P=1.42E-07), and rs17243893 and rs57890595 (HRinteraction 0.51, 95% CI 0.3-0.6, P=2.19E-05). Based on in-silico functional analyses, we hypothesize that the rs5996080 and rs7973914 may affect the BAFFR and TNFR1/TNFR3 receptors and breast cancer survival possibly by disturbing both the canonical and the non-canonical NF-κB pathways or their dynamics, whereas rs17243893 and rs57890595 interaction on patient survival may be mediated through TRAF2 and TRAIL-R4 interplay. These results warrant further validation and functional analyses. BCAC is funded by Cancer Research UK [C1287/A10118, C1287/A12014] and by the European Community´s Seventh Framework Programme under grant agreement number 223175 (grant number HEALTH-F2-2009-223175) (COGS).

C04.3
Towards understanding the genomic architecture of cancer genomes

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Understanding the genetic architecture of cancer requires genomic and integrative approaches. Cancers feature genomic alterations ranging from single-base changes to large-scale structural variation (SV) involving gains and losses, and rearrangements. Having a complete catalogue of mutations in cancer is crucial for identifying key drivers and providing accurate diagnosis, prognosis, and targeted therapy. Whole-genome sequencing has become more routine and affordable since the introduction of next-generation sequencing (NGS) technologies. However, NGS platforms have limited power to decipher large, complex structural variants frequently observed in cancer. Genome mapping represents a complementary technology that provides critical structural information. It involves high throughput analysis of single molecules spanning hundreds of kilobases in nanochannels. Long-range information is preserved and direct interrogation of complex structural variants made possible.

Here, we present our analysis of well-studied and highly rearranged cancer genomes such as the near-tetraploid HCC1143 cell line. We constructed completely de novo genome map assemblies with N50 lengths of more than 1 Mb. We derived multi-sample normalized copy number profiles of matched tumor-control pairs based on genome mapping data. We observed that tumor samples had highly variable copy number profiles, corresponding to focal and chromosome-scale changes. We also present a pipeline to integrate NGS and genome mapping data to validate and refine translocation calls. Genome mapping data helped bridge and phase neighboring translocation events. Finally, we present a computational approach to identify translocations by clustering single molecules with abnormal alignment to the reference and by performing local assemblies of these molecules. Overall, integrating NGS and genome mapping data provides a comprehensive view of a cancer genome.

C04.4
Molecular classification of diffuse cerebral gliomas using genome- and transcriptomewide profiling.

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Cerebral gliomas of World Health Organization (WHO) grade II and III represent a major challenge in terms of histological classification and clinical management. Here, we characterize genomic and transcriptional profiles in a prospective patient cohort of the German Glioma Network. We performed microarray-based genome- and transcriptome-wide profiling of 137 primary glioma samples, including 61 WHO grade II and 76 WHO grade III tumors. Integrative bioinformatic analyses were employed to define molecular subgroups, which were then related to histology, molecular biomarkers, including *IDH1/2* mutation, 1p/19q co-deletion, *TERT* promoter mutation, and patient outcome. Genomic profiling identified five distinct glioma groups. Expression profiling revealed evidence for eight transcriptionally different tumor groups. Correlation of molecular stratification with clinical outcome defined three major prognostic groups with characteristic genomic aberrations. The best prognosis was found in patients with *IDH1/2* mutant and 1p/19q co-deleted tumors. Patients with *IDH1/2* wild-type gliomas and glioblastoma-like genomic alterations, including gain on 7q, loss on 10q, *TERT* promoter mutation and oncogene amplification, displayed the worst outcome. Intermediate survival was seen in patients with *IDH1/2* mutant but 1p/19q intact, mostly astrocytic gliomas, and in patients with *IDH1/2* wild-type gliomas lacking the +7q/-10q genotype and TERT promoter mu-

tation. This molecular subgrouping stratified patients into prognostically distinct groups better than histological classification. Addition of gene expression data to this genomic classifier did not further improve prognostic stratification. In summary, molecular profiling of cerebral gliomas distinguishes biologically distinct tumor groups and provides prognostically relevant information beyond histological classification and *IDH1/2* mutation status.

C04.5

Vaccination with monocyte-derived dendritic cells in Lynch syndrome patients: vigorous T cell responses to neoantigen frameshift-derived peptides.

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BACKGROUND: Mismatch repair (MMR) deficiency in tumor DNA causes shifts in the translational reading frame resulting in the production of altered peptides. Frame-shift peptides (FSP), such as Caspase-5 and TGF-bRII, are considered 'foreign' by the immune system. Dendritic cells are (DC) the professional antigen-presenting cells of the immune system and decisive in inducing immunity. This is the rationale for vaccination with monocyte-derived DC (moDC) loaded with FSPs to stimulate T-cells to combat Lynch syndrome-associated tumors.
PATIENTS AND METHODS: Lynch syndrome CRC patients (n=3) and healthy mutation carriers (n=20) were vaccinated with DC loaded with CEA and FSP MHC class I binding peptides. After each vaccination round (up to 3), antigen-specific CD8+ T cells were assessed in blood and challenged skin. Injection of minute amounts of the DC vaccine resulted in infiltration of immune cells into the skin. Specificity of these lymphocytes was assessed by flow cytometry with tetrameric MHC complexes binding to T cells that recognize the indicated peptides.
RESULTS: In most patients, after moDC vaccinations, both FSP- and CEA-specific CD8+ T-cells were present. Additionally CD8+ T-cells specific for Caspase-5 and CEA were detectable. The functionality of skin infiltrating T-cells was demonstrated by their production of IFN-g upon stimulation with target cells loaded with CEA or one of the FSPs.
CONCLUSIONS: DC vaccination against CEA and FSP-antigens appears feasible and immune responses towards Lynch syndrome tumor-specific peptides are induced. Our data emphasize DC vaccination can enhance the host's antitumor immunity and underline consideration for cancer prevention in Lynch syndrome.

C04.6

Through the looking glass: the reversion of EMT

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Reversible transitions between epithelial and mesenchymal cellular states (EMT/MET) are considered dynamic processes relevant during cancer progression and metastatic spreading. Whereas EMT facilitates the initial steps of tumour cell detachment, promoting migration and invasion, MET is thought to be required for later on colonization at distant sites. However, MET is generally perceived as a process mirroring EMT, devoided of its own signature. We hypothesize that MET entails its own set of novel and/or differentially active molecular circuitries, generating cells with features distinct from the original epithelial state.
Using an in vitro TGFβ1-induced EMT/MET model, we demonstrate that MET is able to generate a heterogeneous population of cells (Reverted-Epithelial or RE cells) with a biological signature not necessarily mirroring that of EMT. These RE-cells displayed novel functional properties, such as increased self-renewal potential and in vivo tumourigenesis ability. Moreover, whole-transcriptome analysis revealed de novo activation of several pathways, such as Toll-like receptor signalling, further confirmed in RE-cells-derived tumours. Considering the increasing evidences towards tumours as a heterogeneous entities, our model is a valuable tool for the discovery of novel pathways of relevance for tumour progression.
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C05.1

MFAP5 loss-of-function mutations underscore the involvement of matrix alteration in the pathogenesis of Familial Thoracic Aortic Aneurysms and Dissections

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Thoracic aortic aneurysm and dissection (TAAD) is an autosomal-dominant disorder with major life-threatening complications. Thedisease displays great genetic heterogeneity with some forms allelic to Marfan and Loeys-Dietz syndrome, and an important numberof cases still remain unexplained at the molecular level. Through whole-exome sequencing of affected members in a large TAAD-affectedfamily, we identified the c.472C>T (p.Arg158*) nonsense mutation in MFAP5 encoding the extracellular matrix component MAGP-2.This protein interacts with elastin fibers and the microfibrillar network. Mutation screening of 403 additional probands identifiedan additional missense mutation of MFAP5 (c.62G>T [p.Trp21Leu]) segregating with the disease in a second family. Functional analysesperformed on both affected individual's cells and in vitro models showed that these two mutations caused pure or partial haploinsufficiency.Thus, alteration of MAGP-2, a component of microfibrils and elastic fibers, appears as an initiating mechanism ofinherited TAAD.
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C05.2

Mutations in a TGFβ ligand, TGFB3, cause syndromic aortic aneurysms and dissections

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Thoracic aortic aneurysms (TAA) are a common condition associated with high mortality due to aortic dissection or rupture. Investigations of the pathogenic mechanisms involved in syndromic types of TAA such as Marfan (MFS) and Loeys-Dietz syndromes (LDS), have revealed an important contribution of disturbed TGFβ signaling. Genes with mutations leading to TAA syndromes include FBN1, TGFBR1/2, SMAD3, TGFB2 and SKI.
To discover novel genes causing syndromic aortic aneurysms, we combined genome wide linkage analysis, whole exome sequencing and candidate gene Sanger sequencing in a total of 470 index cases with thoracic aortic aneurysms. Extensive clinical, cardiologic and imaging examinations were performed.

Presently, we report on 43 patients in 11 families with syndromic presentations of aortic aneurysms caused by mutations in the TGFB3 gene. We demonstrate for the first time that TGFB3 mutations are associated with significant cardiovascular involvement, including thoracic/abdominal aortic dissection and mitral valve disease. Other systemic features overlap clinically with LDS, MFS and Shprintzen-Goldberg syndrome, including cleft palate, bifid uvula, skeletal overgrowth, cervical spine instability and club foot deformity. In line with previous observations in aortic wall tissues of patients with mutations in effectors of TGFβ signaling (TGFBR1/2, SMAD3 and TGFB2), we confirm a paradoxical upregulation of both canonical and non-canonical TGFβ signaling in association with upregulation of expression of TGFβ ligands.
Our findings emphasize the broad clinical variability associated with TGFB3 mutations and highlight the importance of early recognition of the disease due to high cardiovascular risk.

C05.3

Exome-chip meta-analysis identifies novel associations of coding variants with cardiac conduction in 62,251 adults of European descent from the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium.

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Introduction: Electrocardiogram measured QRS interval reflects ventricular depolarization and conduction time. Prolonged QRS is associated with increased risk of sudden cardiac death and heart failure. In previous work, we have identified common variants in loci associated with QRS interval duration comprising non-coding regions of the genome. We hypothesized that genes influencing this trait may harbour both common and rare variants in protein-coding regions.
Methods: The Illumina HumanExome Beadchip (Ex-Chip) is an array focused on rare and low frequency putative functional coding variation. We conducted an Ex-Chip meta-analysis for QRS interval duration in 62,251 participants from 17 studies collaborating in the CHARGE Ex-Chip EKG Consortium. Ex-Chip significance threshold was set at 2.37x10-7 (α=0.05/211,270 polymorphic markers passing QC) for single variant association analysis and 3.06x10-6 (α=0.05/16,326 genes with more than 1 polymorphic marker passing QC) for gene-collapsed variant analyses (SKAT).
Results: In addition to confirming previously associated loci, we identified 10 new loci associated with QRS duration, and provide suggestive evidence for very rare coding variants in previously unknown QRS loci in ADAMTS6, ARID1B, FHOD3 and KRT15 (P<1x10-5, MAF ~0.0021-0.000016).
Using SKAT we identified four novel genes harbouring uncommon and rare variants associated with QRS duration (PSKAT<3.06x10-6).
Conclusion: This is the largest genetic association study for QRS duration performed thus far. Replication studies are ongoing to validate the novel loci. Our study provides greater understanding of the genetic contribution to cardiac conduction and may ultimately lead to new approaches to diagnose, treat, and prevent SCD in humans.

C05.4

A genome-wide association study of nonsyndromic mitral valve prolapse and functional studies of risk loci provide insight into underlying biological mechanisms

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Background. Nonsyndromic mitral valve prolapse (MVP) is a common (2-4%) degenerative valvulopathy, a risk factor for cardiac failure and the most frequent indication for surgical repair of mitral regurgitation. We performed a genome-wide association study to identify risk loci, which may uncover novel pathogenic mechanisms.
Methods. We tested 4.8 million common genotyped/imputed variants in 1412 MVP cases and 2439 controls. Replication for 23 loci was performed in 4 case control studies (Ncases=1442, NControls=6779). Candidate genes were investigated for protein expression by immunohistochemistry on valves in embryonic and adult mice and morpholino knockdown (KD) assessed cardiac function in zebrafish.
Results. Six susceptibility loci were identified after replication (P<5×10-8). Association was observed in LMCD1 (OR=1.32, P =1.3×10-11), a repressor of GATA6 previously implicated in cardiac hypertrophy. KD of Lmcd1 in zebrafish induced significant atrioventricular (AV) valve defect with regurgitation. Another signal on Chr2q35 (OR=1.25, P=3.1× 10-11) mapped upstream to TNS1, which encodes a focal adhesion and actin interacting protein. We found that tensin1 is expressed during valve morphogenesis in mice and maintained in the adult valvular interstitial cells. Nine months Tns1-/- mice exhibited enlarged posterior mitral leaflets compared to wild type. In addition, zebrafish KD of Tns1 induced AV regurgitation.
Conclusions. In this multidisciplinary study we discovered 6 loci with moderate effect sizes (OR range: 1.22-1.33) and identified new actors in valve development and biology. We provide genetic and functional evidence implicating LMCD1 and TNS1 in valve development and function for the first time. This study reveals new pathways that can potentially be modified to improve the natural history of MVP.

C05.5

Recessive mutations in matrix metallopeptidase 21 (MMP21) cause heterotaxy in humans

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Human heterotaxy syndrome results from a failure to establish normal left-right asymmetry early in embryonic development and comprises visceral malformations among which congenital heart defects (CHDs) are the major cause of morbidity and mortality. Mutations in several genes controlling early left–right patterning have been implicated in heterotaxy but account for a minority of cases.
We performed whole exome or genome sequencing in 2 families with recurrence of complex CHDs associated with laterality defects of abdominal organs. We identified compound heterozygous mutations (stop and missense or frameshift and exonic deletion) in matrix metallopeptidase 21 (*MMP21*) in both families. MMP family members are involved in extra-cellular matrix turnover. Interestingly, mice homozygous for ENU-induced missense mutations in *Mmp21* exhibit CHDs and heterotaxy. Also, we performed knockdown of *mmp21* in zebrafish using an antisense morpholino, which resulted in abnormal cardiac looping, a consequence of disrupted left-right patterning. We then performed next generation sequencing of *MMP21* in a cohort of 168 index cases with CHDs and heterotaxy. From this cohort we identified 9 families with one or more affected siblings exhibiting variations in *MMP21* on both alleles, including a homozygous missense affecting the start codon in one family and a homozygous frameshift in another. The frequency of *MMP21*-associated heterotaxy in this cohort is therefore potentially as high as 6%, pending the validation of all missense mutations in functional assays in zebrafish. Our results indicate that *MMP21* is a novel gene implicated in heterotaxy and CHDs and suggest that the regulation of the extra-cellular matrix is key for the establishment of laterality.

C05.6

Somatic/mosaic mutations are an important cause of sporadic vascular anomalies.

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Vascular anomalies are localized defects of the lymphatic or vascular system. They are most commonly cutaneous, but can affect any body part. They are divided according to vessel type into arterial, capillary, venous, lymphatic and combined malformations.

Based on familial inheritance of some malformations, we have identified underlying germline changes, including in TIE2 (mucocutaneous venous malformations; VMCM), in glomulin (glomuvenous malformations; GVM), and RASA1 (capillary malformation-arteriovenous malformation; CM-AVM). These patients are characterized by small multifocal lesions, which may increase in number with time. We explained this by Knudson's double-hit theory and demonstrated somatic second-hits (intragenic or chromosomal anomalies, including acquired uniparental isodisomy) in all three entities.
The frequency of somatic 2nd-hits made us study sporadic patients for somatic changes. We screened resected tissues from >100 venous (VM), >70 lymphatic (LM) and >30 capillary (CM) malformations for mutations in candidate genes, either by RT-PCR or Ion Torrent PGM, for high sensitivity. We frequently pinpointed somatic activating mutations: in +/-50% of VMs in TIE2, 80% of LMs in PIK3CA, 50% of CMs in GNAQ, and in some syndromic forms, like CLOVES syndrome, in PIK3CA. Interestingly, sporadic patients with multifocal VMs were mosaic in blood with a somatic second-hit in tissues, whereas in Blue Rubber Bleb Nevus syndrome, somatic cis-mutations in distant lesions were shared, without blood mosaicism.
We conclude that somatic mutations are a common pathophysiologic cause of sporadic vascular anomalies. Wider mosaicism is evident in sporadic patients with multifocal lesions. Yet, migration of mutant "progenitor cells" underlies multifocality in others. Similar scenario may hold true for various other disorders.

C06.1
Neurogenetic disease diagnostics by targeted capture and next generation sequencing

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Introduction: Two problems for molecular diagnosis of neurogenetic disorders are high levels of genetic heterogeneity and involvement of large genes, e.g. titin.
Materials and Methods: To offset these problems we developed a TargetSeq (Life Technologies) capture panel containing 277 genes in which mutations detectable by next generation sequencing (NGS) cause neurogenetic diseases. The diseases range from foetal akinesia, through muscular dystrophies and peripheral neuropathies, to motor neuron disease. The targeted genes were captured from pooled bar-coded patient DNA samples. Up to 24 patient samples were sequenced at a time using Ion Proton™ (Life Technologies) sequencing. Variant analysis was by either custom Annovar or Cartagenia (Cartagenia, Inc.) based pipelines.
Results: Testing the panel identified 88% of the known small-scale mutations in 28 positive controls. Known mutations deliberately chosen in regions not sequenced well by NGS were not detected. Using FishingCNV, we identified 90% of CNV controls, including 100% of CMT1A/HNPP duplication/deletion controls. Although one single exon CNV was detected, not all small CNVs were detected. We have screened approaching 600 molecularly undiagnosed probands, whose clinical diagnosis matched the diseases screened by the panel. We identified mutations in 68 genes, 40 of which were not previously analysed at all by the diagnostic laboratory due to Sanger sequencing costs. The overall success rate (Class 5 and 4 variants) was 34%, varying from 20% in distal arthrogryposis and SMA/MND, to 57% in channelopathies.
Conclusions: It is remarkable that analyzing only 277 genes provides the molecular diagnosis for 34% of the spectrum of neurogenetic patients.

C06.2
The SMCHD1 mutation spectrum in Facioscapulohumeral muscular dystrophy

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Facioscapulohumeral muscular dystrophy (FSHD) predominantly affects the muscles in the face, trunk and upper extremities. FSHD is associated with partial chromatin relaxation of the D4Z4 macrosatellite repeat array localized on chromosome 4 and transcriptional derepression of the D4Z4-encoded *DUX4* gene in skeletal muscle. In the most common form, FSHD1, this D4Z4 chromatin relaxation is caused by a D4Z4 repeat array contraction to 1-10 units (normal range 10-100 units). In the rare form of FSHD, FSHD2, D4Z4 chromatin relaxation occurs without repeat contraction, and is most often caused by loss of function mutations in the structural maintenance of chromosomes hinge domain containing 1 (*SMCHD1*) gene. SMCHD1 is involved in the maintenance of D4Z4 methylation and a repressed chromatin structure.
An *SMCHD1* mutation screen in a large cohort of FSHD2 individuals revealed a mutation spectrum that ranges from large deletions causing *SMCHD1* hemizygosity, to missense, nonsense and splice site mutations. In addition to the autosomal dominant inheritance seen in most FSHD2 families, we also identified a family with semidominant inheritance. Furthermore, *SMCHD1* mutations can also modify disease severity in FSHD1.
This mutation spectrum shows that epigenetic sensitivity to disease presentation depends on the nature of *SMCHD1* mutation in combination with *D4Z4* array size with *SMCHD1* open reading frame preserving mutations being more deleterious for the maintenance of repressive D4Z4 chromatin state, than open reading frame disrupting mutations. Collectively, our study positions SMCHD1 central in the FSHD2 and FSHD1 disease mechanisms and warrants further studies into SMCHD1 as therapeutic target.
Grant references:
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C06.3
Plastin 3, a human protective modifier is highly upregulated in iPSC-derived motoneurons in asymptomatic individuals and rescues spinal muscular atrophy in mice

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Spinal muscular atrophy (SMA) is a devastating motoneuron disorder caused by functional loss of SMN1. Previously, we identified Plastin 3 (PLS3) as a strong candidate protective modifier using transcriptome differential expression analysis in SMA discordant families.
PLS3 was highly upregulated in lymphoblastoid cell lines of asymptomatic but not SMA siblings at both, RNA and protein level. Instead fibroblast cell lines from both SMA and asymptomatic siblings showed similar PLS3 expression, suggesting a tissue-specific regulation. To investigate whether PLS3 is upregulated in motorneurons, the primary affected cells in SMA, we generated induced pluripotent stem cells (iPSCs) from fibroblasts of three asymptomatic and three SMA III-affected siblings. After full characterization of pluripotency, small molecule neural precursor cells (smNPCs) were generated from iPSCs. Next, motoneurons were differentiated from smNPCs and characterized for any possible changes including survival, gem counts, protein and RNA expression of SMN and PLS3. Most strikingly, PLS3 was highly upregulated only in motoneurons from asymptomatic siblings pinpointing a tissue-specific regulation.
To finally address the PLS3 rescuing effect in SMA disorder, we generated PLS3 overexpressing mice, which were crossed into a severe SMA mouse model. By applying low amounts of exon 7 inclusion SMN antisense oligonucleotides (ASOs), we improved the multi-organ dysfunction in these severe SMA mice and increased their survival from 14 to 28 days. Most importantly, crossing the PLS3 transgene homozygously into these mice led to a robust increase of survival (>60% survived >180 days). These combined data provide strong evidence for PLS3 as protecting modifier in humans and SMA mouse model.

C06.4
Analysis of the Gdap1 knockout mice reveals calcium homeostasis and mitochondrial dynamics defects in the Charcot-Marie-Tooth disease pathogenesis

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Definition of pathogenic mechanisms and druggable molecular targets in Mendelian diseases requires investigating the pathophysiological consequences of gene mutations in both cellular and animal disease models.
Mutations in *GDAP1*, which encodes a protein located in the mitochondrial outer membrane, cause axonal recessive (AR-CMT2), axonal dominant

(CMT2K) and demyelinating recessive (CMT4A) forms of Charcot-Marie-Tooth (CMT) neuropathy. Loss of function recessive mutations in *GDAP1* are associated with decreased mitochondrial fission activity, while dominant mutations result in impairment of mitochondrial fusion with increased production of reactive oxygen species and susceptibility to apoptotic stimuli.
Knockout *Gdap1* mice show abnormal motor behaviour at early stage. Electrophysiological and biochemical studies confirmed the axonal nature of the neuropathy whereas histopathological studies showed progressive loss of motor neurons (MNs) in the anterior horn of the spinal cord and defects in neuromuscular junctions. Cultured embryonic MNs neurons showed large and defective mitochondria, changes in the endoplasmic reticulum (ER) cisternae and increased autophagy vesicles. We observed defects in cytoskeletal α-tubulin acetylation and in the axonal mitochondria transport. MNs showed reduced Ca^{2+} inflow through store-operated Ca^{2+} entry (SOCE) upon mobilization of ER-Ca^{2+}, in association with an abnormal distribution of the mitochondrial network when treated with the ER stress inducer thapsigargin.
The phenotypic and functional study of the *Gdap1* KO mice revealed the presence of an axonal neuropathy. We propose that lack of GDAP1 induces changes in the mitochondrial network biology and mitochondria-endoplasmic reticulum interaction leading to abnormalities in calcium homeostasis, which may represent part of the *GDAP1*-related CMT pathophysiology.

C06.5
Junctophilin-1 expression levels could modify the effects of GDAP1 mutations in Charcot-Marie-Tooth disease

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Charcot-Marie-Tooth (CMT) disease is a hereditary sensory and motor neuropathy with more than 60 genes associated. CMT type 2K (CMT2K) is caused by mutations in the GDAP1 gene and is characterized by incomplete penetrance and intrafamilial clinical variability. We have recently described the junctophilin1 (JPH1) as a genetic modifier of GDAP1. We characterized the combination of the JPH1 p.R213P and the GDAP1 p.R120W mutation in one patient with a more severe clinical picture. Through cellular studies we established that the combination of these two mutations significant increases the basal cytosolic Ca2+ and reduces SOCE activity, and therefore, JPH1 contribute to the phenotypical consequences of GDAP1 mutations.
Junctophilin genes are characterized by having a long 3'UTR (from 1861 nt of JP in Drosophila melanogaster to 2347 nt of JPH1 in humans) and that is conserved in the case of JPH1. We searched for variants in the 3'UTR of JPH1 in CMT2K families with the GDAP1 p.R120W mutation. We have identified the ENST00000342232.4:c.*1962G>A (rs57375187) variant in two brothers with an unusual early onset and severe clinical picture. We have demonstrated that the c.*1962G>A increase the transcript levels by a luciferase assay. Moreover, with the aim to gain insight into the disease mechanisms, we have used a Drosophila models in order to investigate how altered junctophilin expression levels could modify the effects of the Gdap1 related neural degeneration. Moreover, the Drosophila model has allowed us to discover new pathways related to junctophilin.
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C06.6
CCDC174 mutation underlies a syndrome of hypotonia and psychomotor developmental delay with abducens nerve palsy

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Two siblings of non-consanguineous Ethiopian Jewish ancestry presented with congenital axial hypotonia, weakness of the abducens nerve, psychomotor developmental delay with brain ventriculomegaly and variable thinning of corpus callosum, cardiac septal defects and undescended testes. Homozygosity mapping identified a single disease-associated locus of 3.5Mb on chromosome 3. Studies of a Bedouin consanguineous kindred with 4 individuals affected with a similar recessive phenotype identified a single disease-associated 18Mb homozygosity locus containing the entire 3.5Mb locus of the Ethiopian family. Whole exome sequencing demonstrated one homozygous mutation in CCDC174 (c.1404A>G, p.[*468Trpext*6]) within a shared identical haplotype of 0.6Mb, common to both Bedouin and Ethiopian affected individuals, suggesting an ancient common founder effect. The mutation segregated as expected in both kindreds and was not found in 400 Bedouin and 100 Jewish Ethiopian controls. Knockdown of the CCDC174 ortholog in Xenopus laevis embryos resulted in poor neural fold closure at the neurula stage with later embryonic lethality. Knockdown embryos exhibited a sharp reduction in expression of n-tubulin, a marker for differentiating primary neurons, and of hindbrain markers krox20 and hoxb. The Xenopus phenotype could be rescued by the human normal, yet not the mutant CCDC174 transcripts. CCDC174 is ubiquitously expressed and was previously shown to interact with EIF4a3 which is crucial for RYR1 formation in frogs. In accordance, while maintaining normal mRNA level, RYR1 proteins in skeletal muscle of our patients were not detectable by immunohistochemistry. Also, in-vitro model showed co-localization of CCDC174 and EIF4a3 in the nucleus while overexpression of mutant but not wild-type CCDC174 caused rapid cell death.

C07.1
Does paternal imprinting of FOXF1 on 16q24.1 explain maternal UPD(16) phenotype?

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Trisomy 16 in humans, typically resulting from maternal meiosis I nondisjunction, is the most common prenatal trisomy and lethal unless rescued early embryonically. In one-third of such cases, children with maternal UPD(16) manifest IUGR (attributed to trisomic placenta) and multiple congenital malformations, including heart defects, pulmonary hypoplasia, tracheosophageal fistula, gut malrotation, absent gall bladder, renal agenesis, hydronephrosis, imperforate anus, and single umbilical artery. In contrast, relatively normal phenotype was reported in few patients with paternal UPD(16), and imprinted gene(s) on chromosome 16 were suggested as causative for maternal UPD(16) phenotype. All the above clinical features, except IUGR, are observed in the vast majority of children with a neonatally lethal lung developmental disorder Alveolar Capillary Dysplasia with Misalignment of Pulmonary Veins (ACDMPV). ACDMPV is caused by heterozygous point mutations or genomic deletions involving *FOXF1* on 16q24.1, previously shown to be paternally imprinted, incompletely and in a tissue-specific manner. Most recently, genomic duplications involving *FOXF1* were associated with pyloric stenosis, mesenterium commune, and aplasia of the appendix [PMID: 25472632]. We knocked-in a Cre-inducible *Foxf1* allele at the *ROSA26* locus and activated it with *Tie2-cre* to specifically overexpress *Foxf1* in endothelial and hematopoietic cells. Using timed-matings, microCT, and plethysmography analyses, we found that these mice exhibit hypoplastic lungs, abnormal breathing, gastrointestinal abnormalities, edema, skin hemorrhages, and die perinatally. We propose that *Foxf1* overexpression in mice corroborates the clinical observations seen in patients with maternal UPD(16) and conclude that paternal imprinting of *FOXF1* may be responsible for the key phenotypic features of maternal UPD(16).

C07.2
Next-gen cytogenetics in prenatal diagnosis: lessons learned with balanced de novo rearrangements

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Specific identification of disrupted and dysregulated genomic regions is critical in precision diagnosis and in management of some individuals with constitutional and acquired rearrangements, especially considering the accelerated increase in annotation of the human genome. Rapidly defining structural chromosome abnormalities that underlie these genomic regions at the nucleotide level in a genome-wide fashion has become feasible

in recent years with ongoing improvements in sequencing technologies, and is revolutionizing the discipline of cytogenetics. The capability of mapping breakpoints precisely is the foundation of the Developmental Genome Anatomy Project (DGAP, www.dgap.harvard.edu), in which more than 150 subjects with apparently balanced chromosome rearrangements have been sequenced revealing a wide variety of genes in human development. In this exciting era of "Next-Gen Cytogenetics," in which traditional cytogenetic techniques and next-gen sequencing are used synergistically, integration of genomic sequencing into the prenatal diagnostic setting is possible within an actionable time frame. To date, we have participated in the evaluation of 10 prenatal cases of *de novo* balanced translocations and inversions for next-gen cytogenetic analysis, and nine cases are completed. With the exception of a complex case involving multiple chromosome rearrangements, all were interpreted by aCGH analyses to be without clinically significant gains or losses of DNA. Use of convergent genomic evidence and quantitative assessment of transcripts in the breakpoint regions are employed in interpretation of potential pathogenicity. In overview, each case has contributed uniquely to our experience in the evolution of this approach to a new standard of care in prenatal diagnosis.

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C07.3

Targeted prenatal screening as a successful and fast approach in cases with increased nuchal translucency and/or abnormal ultrasound

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Since we have successfully established Next Generation Panel Sequencing of more than 4800 known Mendelian disease genes in postnatal cases resulting in an overall diagnostic yield of more than 45% we investigated the diagnostic yield of targeted Next Generation Sequencing on prenatal cases. Routine PTPN11 sequencing on 53 cases with increased nuchal translucency revealed in 4 (7.5%) pathogenic mutations. Sequencing of the "Mendeliome" in so far 17 cases negative for PTPN11 and setting of specific in silico filters for Noonan genes revealed one novel pathogenic mutation in KRAS (c.149C>T/= (p.Thr50Ile/=) in a case with increased nuchal translucency and multiple heart anomalies, giving a diagnostic yield of about 6% in PTPN11-negative cases. Moreover, an average yield of less than one rare (MAF < 2%) non-synonymous call per case allows a fast and straightforward analysis. An average coverage of 200x and a 20x coverage in almost 96% of the targeted region assures also high sensitivity.

Additional analysis on further prenatal cases with intrauterine growth retardation (2), hydrops fetalis (1), brain malformations (1), fetal bronchial aplasia (1), or uniparental disomy 13 (1) unraveled one new possible disease causing candidate gene in a case with complex brain malformations. Furthermore, screening on 12 aborted samples achieved a diagnosis in 3 (25%) cases, i. e. autosomal recessive pathogenic mutations in the genes FRAS1, MKS1 and CC2D2A.

We conclude that the fast workflow and the high sensitivity makes the approach ideal for targeted screening on prenatal and aborted cases.

C07.4

Comprehensive carrier genetic test using next-generation DNA sequencing in infertile couples wishing to conceive trough assisted reproductive technologies (ART)

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Introduction: Next-generation sequencing (NGS) enables screening for a wide range of conditions that a family history will never pick up. Here, we report the clinical results of a NGS-based preconception carrier genetic test (CGT) for mendelian conditions.

Materials and Methods: Massive sequencing of 548 autosomal recessive (AR) and X-linked genes involved in severe childhood phenotypes reinforced with 5 non-NGS complementary tests, performed in ART patients. In case of gamete donation, paired results (donor/patient) were blindly matched using a proprietary software.

Results: We performed a total of 2,559 CGT analysis: 1,158 tests for gamete donors; 1,127 tests for patients using donation, and 274 CGT for 137 couples undergoing ART with their own gametes. Positive results for at least one pathogenic or likely pathogenic variants were present in 92.5% samples (2,368). The average carrier burden per sample was 2.94 mutations and 14.39 variants of unknown significance. Female donors were discarded in case of pathogenic variant was found in an X-linked gene. In our series 1.5% resulted positive (8 premutated in FMR1 gene and 5 for other X-linked conditions). Twelve couples using their own gametes (8.7%) were positive for pathogenic mutations, 8 for AR genes and 4 X-linked genes. These couples received genetic counseling and PGD or gamete change was recommended.

Conclusions: We have developed a carrier genetic test that constitutes a powerful clinical tool to avoid more than 600 diseases in the offspring in infertile couples undergoing ART.

C07.5

Non-manifesting AHI1 truncations indicate localized loss-of-function tolerance in a severe Mendelian disease gene

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Determination of variant pathogenicity represents a major challenge in the era of high-throughput sequencing. Erroneous categorization may result if variants affect genes which are in fact dispensable. We demonstrate that this also applies to rare, apparently unambiguous truncating mutations of an established disease gene. By whole-exome-sequencing (WES) in a consanguineous family with congenital non-syndromic deafness, we unexpectedly identified a homozygous nonsense variant, p.Arg1066*, in *AHI1*, a gene associated with Joubert syndrome (JBTS), a severe recessive ciliopathy. None of four homozygotes expressed any signs of JBTS, and one of them had normal hearing, which also ruled out p.Arg1066* as the cause of deafness. Homozygosity mapping and WES in the only other reported JBTS family with a homozygous C-terminal truncation (p.Trp1088Leufs*16) confirmed *AHI1* as disease gene, but based on a more N-terminal missense mutation impairing WD40-repeat formation. Morpholinos against N-terminal zebrafish *Ahi1*, orthologous to where human mutations cluster, produced a ciliopathy, but targeting near human p.Arg1066 and p.Trp1088 did not. Most *AHI1* mutations in JBTS patients result in truncated protein lacking WD40-repeats and the SH3 domain; disease was hitherto attributed to loss of these protein-interaction modules. Our findings indicate that normal development does not require the C-terminal SH3 domain. This has far-reaching implications, considering that variants like p.Glu984* identified by preconception screening ("Kingsmore panel") do not necessarily indicate JBTS carriership. Genomes of individuals with consanguineous background are enriched for homozygous variants that may unmask dispensable regions of disease genes, and unrecognized false positives in diagnostic large-scale sequencing and preconception carrier screening.

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C07.6

How to design expanded carrier screening panels? Results of an interview study with European geneticists

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Preconception carrier screening for recessive disorders makes it possible to identify couples who, although unaffected by the disorder themselves, are at risk of having an affected child. Historically, carrier screening has been performed for a set of common pathogenic mutations associated with a limited number of disorders. However, recent advances in genetic technology have enabled a more comprehensive coverage of both disorders and pathogenic variants at little additional cost. With the development of expanded carrier screening (ECS) panels that screen for variants associated with hundreds of recessive disorders, it has become increasingly important to devise suitable criteria for the inclusion of disorders and specific pathogenic mutations on

screening panels.
We conducted in-depth interviews with 16 geneticists from 8 EEA countries to investigate their views on the composition of ECS panels. Most participants favored limiting carrier screening to disorders that, due to their severe nature, would clearly justify altering reproductive plans by at-risk couples. Notably, when discussing severity of the disease, some geneticists considered the impact of the disease on the family as a whole, suggesting screening should also be performed for severe conditions with effective yet burdensome therapeutic interventions. There was disagreement on the inclusion of adult-onset and X-linked disorders, with some geneticists favoring screening under certain conditions, while others supported active exclusion of such disorders. Regarding the selection of specific pathogenic mutations, all 16 participants were strongly in favor of limiting screening to the best-studied variants with clearly established genotype-phenotype associations.

C08.1
Context-specific eQTLs identify hormonal effects in obese Finnish men

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Introduction: Obesity is a serious risk factor for cardiovascular and metabolic disease. Considering the steady increase in obesity prevalence and that GWAS variants explain <5% of body mass index variance, gene-environment interactions may contribute to obesity. We hypothesized that expression profiles in adipose tissue may reflect the molecular consequences of environmental changes underlying obesity. Accordingly, in an obese cellular environment, context-specific expression quantitative trait loci (cseQTL) variants might regulate gene expression distinctly from non-obese conditions.
Materials and Methods: We performed eQTL mapping (FDR<1%) using 7,932,277 SNPs with adipose RNA-sequence data on 17,210 expressed genes in 582 men from the Finnish METSIM cohort. We considered the eQTLs only observed in the obese group, but not in the non-obese or overall groups as obese eQTLs (OB), and vice versa for non-obese (NOB). We tested the OB and NOB eQTL genes for replication in an independent METSIM cohort (n=771) using microarray data (FDR<5%).
Results: We discovered 2,450 OB eQTL genes regulated by 28,267 cis (+/-1Mb) variants; and 1,455 NOB eQTL genes regulated by 10,814 variants, respectively. Of these 55% of the OB and 41% of the NOB genes were consistent across RNA-seq and microarrays in independent cohorts. When searching for potential drivers of context-specificity, we observed that estrogen signaling genes were enriched among OB versus NOB eQTL genes (P=0.001), including HSD17B12, which converts estrone into the most active estrogen, estradiol.
Conclusion: OB eQTL genes exhibit genetic-dependent transcriptional regulation mediated by obesity and involve genes in the estrogen pathway as regulators of male obesity.

C08.2
Genetic variants affect expression of nearly all genes, but only in a specific context

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The expression of nearly all genes is subject to genetic regulation. This is a first conclusion from the largest population-based RNA-sequencing project executed so far, including 4,000 total blood mRNA samples from five Dutch biobanks. The large sample size, unprecedented resolution of this study and the comparison with other large population-based studies revealed that SNPs affecting expression (eQTLs) act in a context-dependent manner. Whereas microarray-identified blood eQTLs (Westra et al., Nature Genetics, 2013) replicated well in our study (87%, including 6.8% with opposite allelic direction), eQTLs identified by RNA-seq in lymphocyte cell lines (LCL) (Lappalainen et al., Nature, 2013) replicated less well (78%, including 12% with opposite allelic direction). This wasn't due to differences in data processing as we analyzed all RNA-sequencing data with a common pipeline, but was partly due to differences in cell types. We inferred cell-type-specific eQTLs, and observed that lymphocyte-specific eQTLs replicated well in LCL, but neutrophil-specific eQTLs didn't. We also observed that higher order effects (e.g. differences in proliferation, differentiation or metabolism) can induce, abrogate or inverse eQTLs. We observed several examples where genetic risk factors affect gene expression only in a specific context. We conclude that the effect of SNPs on gene expression depends on the cell and the cellular state. Since eQTL data is not available for every tissue, cell or cellular state (and their specific combinations), predictions of the effect of genetic variation on gene expression should be carefully interpreted.
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C08.3
Pedigree-Associated Genetics and Recent Environment Make Important Contributions to Metabolic Syndrome Traits.

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Genome-wide association studies (GWAS) have successfully identified thousands of loci for a range of human complex traits and diseases but explain only a limited proportion of trait variation. Mixed linear model analyses capture a greater proportion of phenotypic variance than GWAS single-SNP analyses and provide insight into the genetic and environmental architecture of traits. Using this approach we analysed 18 traits related to metabolic syndrome in a Scottish cohort of ~14,000 individuals genotyped for ~550,000 common autosomal SNPs. Trait variation was partitioned into genetic effects associated with SNPs, genetic effects associated with pedigree, shared family environment, shared couple environment and shared full-sibling environment. On average across traits SNP-associated and pedigree-associated genetic effects each explained around half the genetic variance with recently-shared environment of couples accounting for ~10% of the phenotypic variance on average, all of the three being important sources of variation in metabolic syndrome traits. On the other hand, the environment shared largely in the past by members of a nuclear family or by full-siblings, had a limited impact on trait variation. Our findings point to appropriate models to use in future studies as pedigree-associated genetic effects and couple environmental effects have seldom been taken into account in genotype-based analyses. In samples of unrelated individuals pedigree-associated effects cannot be captured, explaining at least part of the missing heritability, but even in such studies it may be necessary to account for unrelated couples included in the sample to appropriately model the variation and hence make accurate predictions and inferences.

C08.4
Genome-wide study for metabolic phenotypes identifies 62 loci and elucidates the metabolic context of LPA in coronary heart disease

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Metabolic phenotypes are highly heritable and have great potential in providing insight into genetic variation influencing both metabolism and complex diseases. We present the largest evaluation of genetic variance in human

metabolism so far. We combined 123 metabolic phenotypes from blood samples of 24,925 individuals from fourteen European cohorts and associated 62 loci with blood metabolite concentrations. 8 of the loci were new. For 15 loci the lead SNP was low frequency, for 8 a coding variant and 22 involved transcription factor binding sites. We showed that two loci, known for Mendelian amino acid metabolism disease with severe neurological manifestations, also harbor low-frequency variants affecting the same circulating amino acid in healthy population samples. Moreover, we show new evidence that the biosynthesis of lipoprotein(a) (Lp(a)), a known coronary heart disease (CHD) biomarker, is associated with very-low-density lipoprotein metabolism and other lipoprotein particles. Causality of metabolite associations was shown with a genetic risk score ($GRS_{Lp(a)}$) for Lp(a), which composed of 18 SNPs independently associated with circulating Lp(a) levels at genome-wide significance in a discovery cohort. $GRS_{Lp(a)}$ SNPs were confirmed in a replication cohort, where $GRS_{Lp(a)}$ explained 45% of Lp(a) variance. Linking $GRS_{Lp(a)}$ to electronic health records of over 17 000 population-based persons showed that the risk score was associated with CHD outcomes but not with any other disease-category. (ICD10:I20-I25 category, P=6.4×10^{-10}, N_{events}=1251, OR=1.28 per one unit increment in $GRS_{Lp(a)}$)). We present a new hypothesis for the biosynthesis of Lp(a) and our results together with previous findings reinforce the observation that *LPA*-targeting treatment has great potential for CHD risk reduction in humans.

C08.5

Systematic identification of downstream trans-effects for 1,300 known disease associated SNPs

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Genetic risk factors identified in genome-wide association studies are mostly non-coding, making it difficult to understand their functional consequences. So far, large-scale trans-eQTL analyses have identified such downstream functional consequences for only 233 SNPs (Westra et al, NG 2013). To increase this, we used methylation-QTL mapping in peripheral blood of 4,000 population based samples from the Dutch BBMRI-NL BIOS consortium. We observed that 1,300 different GWAS SNPs affect methylation of over 10.000 unique CpGs sites in trans (FDR <0.05), representing a six-fold increase in the number of disease-associated SNPs for which downstream functional effects can be detected.

To address the question in what particular biological processes these specific CpGs are involved, we also generated RNA sequencing data for 2,000 of the samples, permitting us to empirically relate CpG methylation to gene expression effects (eQTMs) for over 18,500 CpG sites (FDR <0.05). By using different genomic annotations we could accurately predict (AUC = 0.8) whether these methylation-gene expression relationships were positive (35%) or negative (65%).

By finally integrating the trans-meQTLs and eQTMs and adapting pathway enrichment method DEPICT, we obtained insights in the downstream functional effects of many genetic risk factors: rs3774959 (mapping close to NFKB1 and associated with ulcerative colitis) significantly affects methylation levels of 355 different CpG sites, of which many map within genes of the NF-kappaB cascade.

These results indicate that large-scale meQTL mapping permits discovery of previously unknown downstream molecular effects for many genetic risk factors, and these effects on trans-methylation levels have a clear biological basis.

BIOS (Biobank-based Integrative Omics Study) is a BBMRI-NL Rainbow project.

C08.6

Integrated analysis of human and bacterial genomes in relation to BMI and blood lipid metabolites.

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Increased body mass index (BMI) and blood lipid levels are risk factors for many metabolic diseases. We aimed to investigate the combined effect of gut microbiome and host genetics on human lipid metabolites. We measured LDL, HDL, triglycerides (TG) and total cholesterol (TC) in 893 individuals from a Dutch population cohort LifeLines-DEEP. The microbiome composition was accessed by 16S rRNA gene sequencing. The genotypes of 157 SNPs previously associated to lipid levels were available for all subjects. We developed the 2-part analysis model to account for both binary and qualitative features of microbiome. The variation of lipids explained by host genetics risk and microbiome were estimated using 80% random samples as discovery and 20% as validation set.

After adjusting for age and gender we identified 66 bacterial operational taxonomic units (OTUs) to be associated with BMI, 114 with TG, and 34 with HDL at false discovery rate (FDR)<0.05. No significant associations were detected for LDL or TC at FDR<0.05. Microbiome composition could explain up to 6.0% of the variation in TG level, 4.0% in HDL and 4.5% in BMI, but only 1.5% in LDL level and 0.7% in TC level. We did not observe strong interaction between genetics and microbiome in the respect to human lipid levels. Mendelian randomization analysis suggested that the microbiome is an independent, causal determinant of BMI and lipid levels. Overall, age, gender, genetics and gut microbiome could collectively explain 9.6% variation in BMI, 17.1% variation in TG and 25.9% variation in HDL cholesterol. These results provide rational for developing microbiome-targeting therapy.

C09.1

High yield of causative mutations by whole exome sequencing in selected individuals with childhood cancer

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Childhood cancer predisposition shows extensive genetic heterogeneity with currently over 100 conditions described, and likely many to be identified. Recognition of genetic predisposition in a child with cancer may lead to better treatment choices and surveillance options. We applied whole exome sequencing on germline DNA of children, and their parents, with cancer and at least one of these features: intellectual disability, congenital anomalies, adult type of cancer, a family history for childhood cancer or multiple primary malignancies. All cases remained undiagnosed after consultation by a clinical geneticist and often multiple genetic tests. Analysis of the first 15 patients resulted in a high yield of causative mutations. Three patients carried mutations in the well-known cancer genes *TP53* and *DICER1* (n=2). In three children, exome sequencing revealed syndromes that possibly contributed to their malignancy (*EP300* based Rubinstein Taybi syndrome in a girl with acute myeloid leukemia; *ARID1A* based Coffin Siris syndrome and *ACTB* based Baraitser Winter syndrome in boys with acute lymphatic leukemia (ALL)). In addition, we identified novel candidate genes like a NOTCH1 pathway activating mutation in *MAML2* (p.P319T) and a mutation in *TYK2* (p.P760L) , both in children with two primary ALL occurrences. The latter finding is particularly interesting since we previously found p.G761V in *TYK2* in a patient with recurrent T-ALL. Both mutations were shown to activate STAT signaling, an important pathway in ALL. Our study shows the value of exome sequencing in childhood cancer predisposition, both to facilitate the diagnosis of known syndromes as well as to trace novel cancer susceptibility genes.

C09.2

Integration of somatic and germline exome data to evaluate pathogenicity of rare variants in cancer predisposition genes

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Genes in which germline mutations confer substantial increased risks of cancer are called cancer predisposition genes (CPG). To date characterisation of cancers arising in CPG mutation carriers has been limited as has utilisation of tumor data from CPG mutations carriers to facilitate clinical interpretation of their germline data. To explore this we have downloaded and reanalysed data from 7,632 germline exomes from the 28 cancer types in The Cancer Genome Atlas (TCGA) using the OpEx exome analytical pipeline which has high sensitivity and specificity for indel detection. In the first instance we have focussed on the BRCA1 and BRCA2 germline data. We identified 155 pathogenic BRCA mutations (PMs) in 7,632 samples and 856 rare nonsynonymous (missense) variants (RNVs), which would typically be reported as variants of uncertain significance (VUS). Tumor data of multiple types strongly suggest that the great majority of the RNVs are not pathogenic. For example, the PMs are highly clustered in patients with breast or ovarian cancer whereas the RNVs are equally distributed amongst 28 cancer types. Also the alternate allele frequency of the 155 PMs was significantly

higher in their matched tumor samples, but a similar pattern does not appear to be present for the RNVs (analyses still in process - data will be presented). These data indicate that integration of tumor and germline genetic information may have considerable utility in the interpretation of variants of uncertain significance. This work was funded by the RM/ICR NIHR BRC.

C09.3

Expanding the mutation spectrum and phenotype of Polymerase Proofreading-Associated Polyposis (PPAP): novel and previously reported POLE variants

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Constitutional mutations of the POLE and POLD1 genes, coding for DNA polymerase ε e δ subunits, respectively, have been recently identified in patients with multiple colonic adenomas (MCA) and colorectal cancer (CRC). So far, only few families with this autosomal dominant inherited cancer predisposition, named PPAP, have been reported and the phenotype and prevalence of the condition are not well defined. We therefore investigated a total of 62 patients with MCA (> 10), early onset CRC (EA-CRC) and/or familial CRC for mutations in the POLE and POLD1 exonuclease proofreading domains (aa 278-471 e 304-517, respectively), by direct sequencing of genomic DNA, to verify their frequency and associated clinical characteristics. Patients were APC, MUTYH and MMR mutation-negative. In-silico analyses were performed using Polyphen2, SIFT, Mutation Taster, ClustalOmega, Phyre2, and Chimera 1.6.2.
Five POLE variants were identified in 4/62 patients: p.L424V (which has already been reported as germline mutation) in a family with an autosomal dominant phenotype of Turcot syndrome (multiple polyps associated with gliomas) and cutaneous manifestations (multiple pilomatricomas); p.D392G and p.K425R in a patient with two metachronous CRC and familial CRC; p.S459C in a patient with 3 colorectal adenomas and a positive family history of EA-CRC; p.P436S in a proband with EA-CRC and MCA. Bioinformatic analyses are concordant in predicting a pathogenetic effect for p.K425R, p.S459C and p.P436S, while interpretations of p.D392G are discordant.
Our results contribute to a better definition of the phenotype and mutation spectrum of PPAP caused by POLE defects.

C09.4

Germline mutations in patients with hereditary breast and ovarian cancer establish ERCC2 as a cancer susceptibility gene.

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Background: Breast and ovarian cancer (BC/OC) predisposition is associated with a number of high- and low-penetrance susceptibility genes. Despite comprehensive testing there is still a large portion of high risk cases without mutation in any of the known susceptibility loci. Therefore novel candidate genes need to be screened. Here we report on the results of testing 94 genes in 717 patients from Germany and Lithuania.
Method: Inclusion criteria for the patients in this study were defined by the German Consortium for Breast and Ovarian Cancer. NGS was performed with 150 bp paired end sequencing (Illumina TruSight cancer panel).
Results: In 19.7 % of the patients, BRCA1/2 mutations have been found. Additional 17.9 % of the patients had null-mutations and unclassified missense variants in the BC/OC susceptibility genes ATM, CDH1, CHEK2, NBN, PALB2, RAD51C/D and TP53. Analysis of the non-BC/OC genes on the TruSight-panel identified 4 protein truncating mutations in the „excision repair gene" ERCC2. Additionally we found 20 rare, unclassified missense variations in ERCC2. These variants have a cumulative allele frequency of 2.9% in our BC/OC cohort, which is 14.5-fold overrepresented compared to the "exome aggregation consortium" cohort. Initial functional assays show that at least some of the protein variants (e.g. NM_000400.3:p.Val536Met) have lost their DNA repair ability.
Conclusion: Overrepresentation of deleterious mutations in our cohort defines ERCC2 clearly as a susceptibility gene for BC/OC predisposition. As part of ongoing research, affected individuals with excluded mutations in the known BC/OC predisposition genes should be tested for mutations in ERCC2.

C09.5

Tumour risks and genotype-phenotype-proteotype analysis in ~800 patients with germline mutations in the succinate dehydrogenase subunit genes SDHB, SDHC and SDHD

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Germline mutations in the succinate dehydrogenase subunit genes SDHB, SDHC and SDHD are the most frequent causes of inherited phaeochromocytomas and paragangliomas. Since these genes were identified over a decade ago, genetic testing for mutations in them has become a standard clinical tool for many patients with these, and other, tumour types. However, the lack of information regarding penetrance and phenotypic variability associated with SDHB/C/D mutations hinders optimum clinical management of individuals who are found to have a germline mutation. In order to address these issues we undertook a retrospective survey of 800 individuals (including 401 previously reported) with identified mutations in SDHB/C/D. Analysis of age-related tumour risks according to relevant gene and mutation type (for SDHB and SDHD) provided novel estimates of penetrance and genotype-phenotype correlations. In silico structural prediction analyses were performed to evaluate the functional effects of SDHB and SDHD mutations. Increased knowledge of the molecular basis of phenotypic variability commonly observed in individuals with germline SDHB/C/D mutations will facilitate the development of personalised management protocols based on gene and mutation-specific tumour risks.
We describe the distinct phenotypes of SDHB Ile127Ser and SDHD Pro81-Leu carriers, which can be explained using structural prediction studies and may indicate a need to move towards mutation-specific management plans for these patients.

C09.6

Germline SMAD9 Mutation Destabilizes PTEN: Exome Sequencing Reveals a Novel Susceptibility Gene For Hamartomatous Polyposis and Gastrointestinal Ganglioneuromas

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Hamartomatous polyposis syndromes (HPS) represent a small but appreciable number of the gastrointestinal inherited cancer predisposition syndromes associated with a substantial risk for developing colonic and extracolonic malignancies. We present a unique case of familial juvenile polyposis syndrome associated with gastrointestinal ganglioneuromas of unknown etiology. The patient underwent genetic testing for the known HPS genes (BMPR1A, SMAD4, ENG, PTEN, STK11) but no mutation was detected. Exome sequencing identified a novel germline mutation in SMAD9 resulting in reduced PTEN expression. We subsequently screened 40 JPS patients and 40 CS patients with HPS for SMAD9 mutations but did not detect any with SMAD9 mutations. Our patient, in addition to HPS, had significant ganglioneuromatosis which is rare but is overrepresented in CS patients with germline PTEN mutations. Our functional experiments show that our patient's SMAD9 mutation results in gain-of-function in SMAD8 leading to reduced PTEN mRNA and protein stability, together, yielding a phenotype (ganglioneuromatous polyps) seen more commonly in patients with germline PTEN mutations. Our study suggests that this may be indirectly regulated by miR21expression. We recommend that all patients with a clinical presentation of hamartomatous polyposis especially those with gastrointestinal ganglioneuromas be referred for genetics evaluation.

C10.1
Whole genome sequencing reveals the mutation characteristics in Autism Spectrum Disorder

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Autism spectrum disorder (ASD) is genetically heterogeneous, with evidence for hundreds of susceptibility loci. Previous microarray and exome-sequencing studies have examined portions of the genome in simplex families (parents and one ASD-affected child) having presumed sporadic forms of the disorder. We used whole-genome sequencing (WGS) of 85 quartet families (parents and two ASD-affected siblings), consisting of 170 individuals with ASD, to generate a comprehensive data resource encompassing all classes of genetic variation (including noncoding variants) and accompanying phenotypes, in apparently familial forms of ASD. By examining de novo and rare inherited single-nucleotide and structural variations in genes previously reported to be associated with ASD or other neurodevelopmental disorders, we found that more than two-thirds of the affected siblings carried different ASD-relevant mutations. These siblings with discordant mutations tended to demonstrate more clinical variability than those who shared a risk variant. Our study emphasizes that substantial genetic heterogeneity exists in ASD, necessitating the use of WGS to delineate all genic and non-genic susceptibility variants in research and in clinical diagnostics. We have now sequenced 200 additional ASD families using WGS. We will present our complete data analysis as well as additional progress from this study.

C10.2
Identification of a common set of microRNAs deregulated in Autism Spectrum disorders

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Autism spectrum disorder (ASD) is a neurodevelopmental disease caused by an interaction between genetic vulnerability and environmental factors. MicroRNAs (miRNAs) have emerged as key post-transcriptional regulators and are involved in multiple aspects of brain development and connectivity. Here, using olfactory mucosal stem cells biopsied from living patients, we identified a signature of four miRNAs (miR-146a, miR-221, miR-654-5p and miR-656) commonly deregulated in ASD. This signature is conserved in primary skin fibroblasts and allows discriminating between ASD and intellectual disability samples. Putative target genes of the differentially expressed miRNAs were enriched for pathways previously associated to ASD and altered levels of neuronal transcripts targeted by miR-146a, miR-221 and miR-656 were observed in patients' cells. In the mouse brain, miR-146a displays strong neuronal expression in regions important for high cognitive functions, and we demonstrate that overexpressing miR-146a leads to alteration of neuronal dendritic arborisation. These findings have strong diagnostic implications and emphasize the role of miRNA expression deregulation in the etiology of ASD, opening new opportunities for therapeutic approaches.

C10.3
Rare variants in GABAA receptor genes in Rolandic epilepsy and related syndromes

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Purpose: Mutations in $GABA_A$ receptor ($GABA_A$-R) subunit genes have been described in a range of epilepsy syndromes. Here, we tested whether mutations in 18 genes encoding for $GABA_A$ receptor subunit genes contribute to the etiology of Rolandic epilepsy (RE) or its atypical variants (ARE).
Methods: We performed exome sequencing in 204 European patients with RE/ARE and compared the frequency of $GABA_A$R genes variants with 728 platform matched controls. We functionally assessed nonsynonymous *GABRG2* variants for protein stability, trafficking, postsynaptic clustering and receptor function.
Results: Out of 18 screened $GABA_A$-R genes, we found a significant enrichment of rare variants in the *GABRG2* gene in RE/ARE patients (5/204, 2.45%) when compared to controls (1/723, 0.14%) (OR = 18.07, 95% CI = 2.01 – 855.07, p = 0.0024, p_{corr} = 0.043). We detected a splice variant (c.549-3T>G) in two unrelated patients as well as three nonsynonymous *GABRG2* variations (p.G257R, p.R323Q, p.I389V). Functional analysis of the nonsynonymous variants showed reduced surface expression of p.G257R and decreased GABA-evoked currents for p.R323Q. The p.G257R mutation resulted in reduced palmitoylation, a posttranslational modification crucial for trafficking of proteins to the cell membrane. Enzymatically enhanced palmitoylation levels restored the surface expression of the p.G257R variant γ2-subunit.
Conclusion: The presented statistical association and functional evidence suggest that mutations in the *GABRG2* gene increase risk of RE/ARE. Restoring the impaired membrane trafficking of some *GABRG2* mutations by augmenting palmitoylation levels offers a therapeutic perspective to reverse the pathogenic effect of such mutants.
DFG: LE1030/11-1, BN416/5-1, NU50/8-1, SA434/5-1, FWF: I643-B09, VH-NG-246, ERA-Net NEURON II CIPRESS, NOW: 175.010.2005.011, 911-03-012, and (NGI)/NOW 050-060-810

C10.4
Hyperexcitability or electrical silencing: *de novo* loss- or gain-of-function mutations in *KCNA2* cause epileptic encephalopathy

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Epileptic encephalopathies are a phenotypically and genetically heterogeneous group of severe epilepsies accompanied by intellectual disability and other neurodevelopmental features. Using next generation sequencing, we identified four different *de novo* mutations in *KCNA2*, encoding the potassium channel $K_V1.2$, in six isolated patients with epileptic encephalopathy (one mutation recurred three times independently). Four individuals presented with febrile and multiple afebrile, often focal seizure types, multifocal epileptiform discharges strongly activated by sleep, mild-moderate intellectual disability, delayed speech development and sometimes ataxia. Functional studies of the two mutations associated with this phenotype revealed an almost complete loss-of-function with a dominant-negative effect. Two further individuals presented with a different and more severe epileptic encephalopathy phenotype. They carried mutations inducing a drastic gain-of-function effect leading to permanently open channels. These results establish *KCNA2* as a novel gene involved in human neurodevelopmental disorders by two different mechanisms, predicting either hyperexcitability or electrical silencing of $K_V1.2$-expressing neurons.

C10.5
Cysteine Correction of NOTCH3: exon skipping as a potential therapeutic strategy for CADASIL

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CADASIL is a disabling hereditary vascular dementia and stroke syndrome, occurring worldwide in ~1:50.000 individuals, for which no treatment is available. The disease is caused by stereotyped mutations in NOTCH3, which alter the number of cysteines in one of the epidermal growth factor-like repeat (EGFr) domains of the NOTCH3 protein. This causes toxic NOTCH3 aggregation and accumulation in the (cerebro)vasculature, leading to vascular smooth muscle cell degeneration and a reduced cerebral blood flow. We hypothesized that re-establishing the correct number of 6 cysteines within EGFr may prevent or reduce toxic NOTCH3 aggregation. To accomplish this NOTCH3 'cysteine correction', we use antisense-mediated exon skipping. Based on extensive in silico protein predictions, at least 12 NOTCH3 exons are eligible for this approach, targeting the majority of CADASIL causing mutations. We now have established that this targeted NOTCH3 exon skipping is feasible both in a CADASIL patient derived cell model and in human NOTCH3 transgenic mice. Using 'skip' cDNA constructs, we show that the modified NOTCH3 proteins are expressed at the cell surface and bind to their canonical ligand Jagged1. This indicates that this selected exon exclusion does not abrogate normal NOTCH3 processing and function. The human NOTCH3 transgenic mice which we generated for in vivo studies show progressive cerebrovascular NOTCH3 accumulation, and are therefore a good model to test potential therapies aimed at preventing or reducing NOTCH3 accumulation. Ongoing studies focus on optimizing in vivo exon skipping, to determine the effect of cysteine correction on vascular NOTCH3 accumulation and ultimately the CADASIL phenotype.

C10.6
De novo deleterious genetic variations target a biological network centered on Aβ peptide in early-onset Alzheimer disease

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We hypothesized that de novo variants (DNV) might participate in the genetic determinism of sporadic early-onset Alzheimer disease (EOAD, onset before 65 years). We investigated first by array-CGH and then by whole exome sequencing 14 sporadic EOAD trios. Two patients carried a de novo copy number variation and 7, among the remaining 12, had at least one non-synonymous DNV. Five of these 9 DNV (an APP duplication, a BACE2 intronic deletion, and three nucleotide variants affecting PSEN1, VPS35 and MARK4) targeted a biological network centered on the Amyloid beta (Aβ) peptide. Using appropriate statistical analyses, we showed that this a priori-defined genetic network was significantly enriched in amino acid-altering DNV, compared to the rest of the exome. In addition, we provided evidence of the functional impact of 4/5 DNV targeting this network: the causality of the APP de novo duplication (which is the first reported one) was obvious; the novel PSEN1 variant resulted in exon 9 skipping in patient's RNA, leading to a pathogenic missense at exons 8-10 junction; the VPS35 missense variant led to partial loss of retromer function, which may impact neuronal APP trafficking and Aβ secretion; and the MARK4 multiple nucleotide variant resulted into increased Tau phosphorylation which may trigger enhanced Aβ-induced toxicity. Despite the difficulty to recruit AD trios due to age structures of the pedigrees and the genetic heterogeneity of the disease, this strategy allowed us to highlight the role of de novo pathogenic events, the putative involvement of three new genes in AD genetics and the key role of Aβ network alteration in AD.

C11.1
Mutations in a novel dynein-2 light chain, TCTEX1D2, cause Jeune Asphyxiating Thoracic Dystrophy (JATD) with incomplete penetrance

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Background: JATD is a very rare, autosomal recessively inherited ciliary chondrodysplasia mainly characterised by short ribs resulting and narrow thorax causing a life threatening respiratory phenotype, sporadic polydactyly and variable extraskeletal findings such as renal, hepatic and retinal disease. Known disease causing genes currently explain approximately 80% of the cases. We have recently identified mutations in components of the cytoplasmic dynein-2 complex, including the heavy chain and 2 novel intermediate chains, but not any light chains.
Objectives, methods and results: To define the underlying genetic and molecular basis, we have genetically explored a cohort of over 300 individuals using diverse DNA sequencing techniques including whole exome sequencing, identifying 3 families with loss of function ("null") mutations in *TCTEX1D2*, a new cytoplasmic dynein-2 (IFT-dynein) light chain. We observed an unusual inheritance pattern suggesting incomplete penetrance and in patient cells and *Chlamydomonas*, impairment of retrograde IFT seems milder than in models for other dynein-2 defects and in human, fish and *Chlamydomonas and* loss of *TCTEX1D2* conferred no apparent changes in gross ciliary structure. Our proteomics analysis in *Chlamydomonas* and human proteomic analysis confirmed that TCTEX1D2/Tctex2b represents a component of an intermediate chain/light chain sub-complex within IFT dynein and that in *Chlamydomonas*, the retrograde IFT defect observed is caused by instability of the entire IFT dynein complex.
Conclusion: Together, our results suggest *TCTEX1D2* is an integral component of the IFT-dynein complex in human, zebrafish and that loss of function mutations cause JATD, however its loss may be compensated for under certain conditions.

C11.2
Mutations in DVL1 cause an osteosclerotic form of Robinow Syndrome

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Robinow Syndrome (RS) is a phenotypically and genetically heterogeneous condition that can be caused by mutations in genes encoding components of the non-canonical WNT signaling pathway. In contrast, germline mutations that act to increase canonical WNT signaling lead to distinctive osteosclerotic phenotypes. Here we identify de novo frameshift mutations in DVL1, a mediator of both canonical and non-canonical WNT signaling, as the cause of a subtype of RS with osteosclerosis (RS-OS) in three unrelated individuals. The mutations all delete the DVL1 C-terminus and replace it, in each instance, with a novel, highly basic sequence. We show the presence of mutant transcript in fibroblasts from one individual with RS-OS, and demonstrate unimpaired protein stability with transfected GFP-tagged constructs bearing a frameshift mutation. In vitro TOPFlash assays, in apparent contradiction to the osteosclerotic phenotype, revealed that the mutant allele is less active than the wild type in the canonical WNT signaling pathway. However, when the mutant and wild type alleles are co-expressed, there is a 2-fold increase in canonical WNT activity over that of the wild type construct alone. This work establishes that DVL1 mutations cause a specific subtype of RS, RS-OS, and that the osteosclerosis may be the result of an interaction between the wild type and mutant alleles leading to elevated canonical WNT signaling.

C11.3
Mutations in ZAK cause autosomal recessive split foot malformation in humans and complex hindlimb defects in mice

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Split hand foot malformation (SHFM) is a clinically heterogeneous defect of the central rays of hands and feet.

By using a combination of homozygosity mapping and exome sequencing in a consanguineous Pakistani family, we have identified a c.1103T>G / p.Phe368Cys mutation in ZAK (also known as MLTK) on chromosome 2q31.1 as the cause of a unique autosomal recessive split foot-hearing loss syndrome with highly variable expressivity. Screening of genetically unresolved SHFM cases identified an unrelated Tunisian individual with a homozygous intragenic ZAK deletion. Our biochemical studies using a super negative GFP gel assay show that the SAM domain of ZAK is monomeric. In situ hybridisation in mouse embryos revealed strong Zak expression in the heart as well as in the developing forelimb and hindlimb between embryonic days (E) 9.5 and 11.5. Consistent with a role for Zak/Mltk in cartilage and bone development morpholino oligonucleotide-mediated knockdown resulted in abnormal cartilage development in Xenopus laevis embryos. Finally, CRISPR-Cas mediated complete inactivation of Zak in mice caused lethality at E 9.5 due to severe cardiac malformation. The targeted deletion of the SAM domain in mice however was associated with a complex hindlimb malformation with some animals showing severe clefting of the whole hindlimb including the femur, tibia, fibula and the feet. Furthermore expression analysis of mutant hindlimbs at E 10.5 and 11.5 showed a 60% decrease of Tp63 expression compared to wild type hindlimbs, suggesting a functional link between Zak and p63.

C11.4
Spondyloenchondrodysplasia: The expanding phenotype of TRAP deficiency

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Spondyloenchondrodysplasia is a rare immuno-osseous dysplasia caused by biallelic mutations in *ACP5.* Clinical, molecular and serological data from a total of 22 patients from 17 families will be described, providing an update regarding the skeletal, neurological and immune phenotype in this condition, in which the spectrum of disease continues to widen with increased identification of affected individuals. Of particular note we propose that the OMIM differentiation between Spondyloenchondrodysplasia and Spondyloenchondrodysplasia with immune dysregulation is not required, as we have shown with molecular testing that they represent a continuum of the same disorder. We observed a diverse immune phenotype, frequently including autoimmune thrombocytopenia and systemic lupus erythematosus, and noted a possible increased susceptibility to infection. In the majority of patients tested we detected upregulated expression of type I interferon-stimulated genes, in keeping with the autoimmune phenotype observed and the likely immune regulatory function of the deficient protein: tartrate resistant acid phosphatase. Interestingly, however we identified two mutation positive patients without an upregulation of interferon-stimulated genes, including one patient with significant autoimmune disease, which was controlled by immunosuppressive therapy. This patient may demonstrate a useful therapy for the immune manifestations of Spondyloenchondrodysplasia.

C11.5
Brachyolmia with amelogenesis imperfecta can be caused by a defect in the TGFbeta signaling pathway

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Brachyolmia refers to a heterogeneous group of skeletal dysplasias with a disproportionate short stature with short trunk. Radiographically the disorder is characterized by a predominant involvement of the axial skeleton with variable degrees of vertebral flattening (platyspondyly). Amelogenesis imperfecta (AI) is a defect in enamel formation/mineralization presenting as an isolated anomaly or occuring in association with other anomalies. In 1996, Verloes et al. described an autosomal recessive form of brachyolmia associated with AI (OMIM 601216). Bertola et al. (2009) subsequently published two other families with the combination of vertebral anomalies and enamel defects. Here we report on four additional families with the same phenotype. Three of the eight affected individuals had significant short stature. All patients had variable degrees of platyspondyly, very thin or almost absent enamel in both primary and permanent dentitions. A combined strategy of homozygosity mapping and whole exome sequencing resulted in the identification of recessive hypomorphic mutations in a gene involved in the TGFbeta signaling pathway. We further investigated the gene expression during mouse and tooth development. At E18.5 labeling was restricted to ameloblasts synthesizing enamel matrix proteins, and to odontoblasts. Investigating an available knockout mouse model showed that the mutant mice displayed thin to absent enamel in both incisors and molars, as well as disorganised ameloblasts layer and matrix hereby recapitulating the amelogenesis imperfecta phenotype in our patients. Our study confirms the role of the TFGbeta signaling pathway in both osteogenesis and amelogenesis.
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C11.6
Pentosan Polysulfate: New Mechanistic Insights and Treatment of the Mucopolysaccharidoses

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Pentosan polysulfate (PPS) is manufactured in both oral and injectable forms, and has been used clinically for over 40 years. Due to the i) anti-inflammatory properties of PPS, ii) positive effects in arthritic animals and patients, and iii) extensive safety history, we investigated the use of PPS in the mucopolysaccharidoses (MPS). Comparative studies of oral (daily) and injectable (weekly subcutaneous, s.c.) PPS were carried out in MPS VI rats (Schuchman et al., 2013; Frohbergh et al., 2014). Both studies led to reduced inflammatory markers, improved dentition and skull lengths, reduced tracheal deformities, and markedly improved mobility. Unexpectedly, a significant reduction of urine and tissue glycosaminoglycans (GAGs), was most evident after s.c. administration. Findings were confirmed by analysis of total GAGs and by mass spectrometry. The mechanism(s) leading to PPS-mediated GAG reduction in MPS are currently being investigated. Further preclinical studies were carried out in MPS I dogs, with oral (daily) and s.c. (every other week) PPS for >1 year. Significant reductions of inflammatory markers, tissue and urine GAGs were most evident with s.c. administration. Both treatment groups also exhibited reduced carotid and aortic inflammation with the absence of plaque formation, a common pathology in the vasculature of MPS I dogs and patients. Based on these findings, two clinical studies evaluating s.c. PPS have been initiated, in adult MPS I (Germany) and MPS II (Japan) patients. We conclude that PPS may be beneficial for MPS, either as an adjunct therapy or as a stand alone treatment that reduces inflammation and GAG storage.

C12.1
A novel disorder reveals Clathrin Heavy Chain-22 is essential for human pain and touch development

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A congenital inability to feel pain is very rare but the identification of causative genes has yielded significant insights into pain pathways and also novel

targets for pain treatment. We report a novel recessive disorder characterised by congenital insensitivity to pain, inability to feel touch, and cognitive delay. Affected individuals harboured a homozygous missense mutation in CLTCL1 encoding the CHC22 clathrin heavy chain. The mutation p.E330K renders CHC22 non-functional in mediating endocytosis at the cell membrane. We found that CLTCL1 is significantly upregulated in the developing human brain, displaying an expression pattern suggestive of an early neurodevelopmental role. Guided by the disease phenotype, we investigated the role of CHC22 in two human neural crest differentiation systems; human iPSC derived nociceptors and TRKB dependant SH-SY5Y cells. In both there was a significant down-regulation of CHC22 upon the onset of neural differentiation. Furthermore, knockdown of CHC22 induced neurite outgrowth in neural precursor cells, which was rescued by stable overexpression of siRNA resistant CHC22 but not by mutant CHC22. Similarly, overexpression of wild-type, but not mutant, CHC22 blocked neurite outgrowth in cells treated with Retinoic Acid. Using a novel, quantitative proteomics approach we have identified the protein complement of clathrin coated vesicles in neuronal cells, and present insights into the neural-specific trafficking defects resulting from loss of functional CHC22. These results reveal an essential and non-redundant role for CHC22 in neural crest development and in the genesis of pain and touch sensing neurons.

C12.2

Exome sequencing of ataxia-blindness patients identifies atypical Brown-Vialetto-Van Laere syndrome-2 (BVVLS2) presentation and identifies PEX6 as the SCAR3 (MIM#271250) gene

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We reported in 2000 the linkage of spinocerebellar ataxia with blindness and deafness (SCAR3) to chromosome 6p23-p21 from the study of a single multigenerational consanguineous family (Bomont et al.). We have now performed exome sequencing of this family and found in all patients the homozygous p.Gly306Arg missense mutation in SLC52A2, previously reported in patients with BVVLS2, but located on chromosome 8qter. High recombination rate in the telomeric region and use of widely spaced microsatellite markers explains why correct linkage was initially missed. Exome sequencing of a second family with two children affected with progressive ataxia, bilateral optic atrophy and mild mental retardation revealed the novel homozygous p.Pro134Leu missense mutation in SLC52A2, confirming that treatable (by riboflavin supplementation) BVVLS2 should be considered for differential diagnosis of syndromic autosomal recessive ataxia. Exome sequencing of a third family with two children affected with progressive ataxia and retinitis pigmentosa and with linkage to 6p23-p21 revealed the p.Ala912Val mutation in PEX6. Retrospective analysis of peroxisomal markers showed just above normal serum phytanic acid levels but fibroblasts from a subsequent skin biopsy revealed absent peroxisomal catalase immunostaining, peroxisomal ghosts with abnormal structure and markedly increased C26/C22 ratio, confirming the pathogenicity of the p.Ala912Val mutation. Therefore, despite initial false linkage, the SCAR3 locus is confirmed and corresponds to PEX6 mutation. Identification of these hypomorphic missense mutations lends further support to the concept that numerous recessive ataxias are caused by partial loss of function mutations in a large variety of pathophysiological pathways (Anheim et al., NEJM, 2012).

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C12.3

Heimler Syndrome is caused by unique hypomorphic mutations in the peroxisome biogenesis genes PEX1 and PEX6

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Heimler syndrome (HS) is a rare recessive disorder characterized by sensorineural hearing loss, enamel hypoplasia, retinal pigmentation and nail abnormalities, for which no gene has been identified. We performed whole exome sequencing in eight families with HS and identified biallelic mutations in PEX1 or PEX6, in six affected families. Loss of function mutations in both genes are known to cause peroxisome biogenesis disorders (PBDs), such as Zellweger syndrome, which is a group of autosomal recessive disorders, characterized by developmental brain abnormalities, sensorineural hearing loss, retinopathy, and skeletal, craniofacial, and liver abnormalities. We analyzed plasma, erythrocytes and cultured skin fibroblasts from affected individuals for biochemical peroxisomal parameters but did not detect any significant aberrations. However, immunofluorescence microscopy of the fibroblasts revealed a so-called mosaic peroxisomal pattern compatible with a very mild peroxisomal dysfunction. The impaired peroxisomal biogenesis in the PEX1 or PEX6 mutant cells could be rescued by transfection of PEX1 or PEX6 cDNA respectively and by functional expression studies we demonstrated which of each mutant allele caused the mild mutant phenotype. Most of these mutations had not been reported previously in PBD patients.

Although individuals with HS share subtle clinical features found in PBDs, the overlap is minimal and the diagnosis was not suggested by routine plasma analyses used to detect PBDs. In conclusion, our findings illustrate the diagnostic utility of exome sequencing for rare syndromes and expand the phenotypic spectrum associated with PEX mutations.

C12.4

An in-frame deletion in *FOXL1* identifies the first gene causing autosomal dominant otosclerosis

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Introduction: Otosclerosis, the most common cause of conductive hearing loss in adults, is characterized by the cementing of the stapes bone to the oval window, and is believed to be caused by dysregulation of bone remodeling in the otic capsule. Although considered sporadic, 10 distinct genetic loci (*OTSC1-10*) have been mapped in families with autosomal dominant forms, but no genes have been identified.

Materials and Methods: We used a combination of linkage, fine mapping and exome sequencing in a multiplex AD family from the island of Newfoundland, Canada.

Results: We identified a 15 bp in-frame deletion in the FOXL1 gene (c.976_990het_delGGGATCCCCTTCCTC) co-segregating in all relatives with surgically confirmed otosclerosis. This in-frame deletion in FOXL1 is predicted to cause the removal of 5 amino acids from the highly conserved C-terminus (NM_005250). Screening of >100 patients from Canada and Europe revealed a second family from Ontario, Canada, with the identical mutation in FOXL1 and shared ancestral haplotype.

Conclusions: Expression studies using human cells suggest that mutant FOXL1 causes dysregulation of cytokines, supporting the prevailing hypothesis that patients with otosclerosis undergo activated bone remodeling in the otic capsule. Interestingly, although not present in ethnically matched controls, the novel c.976_990het_delGGGATCCCCTTCCTC deletion in FOXL1 was recently reported at a frequency of 0.16% in Europeans and 0.09% in Africans. In summary, we report the identification of the first otosclerosis gene.

C12.5

Submicroscopic deletions at 13q32.1 cause congenital microcoria

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Congenital microcoria (MCOR) is a rare autosomal dominant disorder characterized by inability of the iris to dilate owing to absence of dilator pupillae muscle. So far, a dozen MCOR families are reported worldwide. By using whole-genome oligonucleotide array CGH, we have identified deletions at 13q32.1 segregating with MCOR in six families originating from France, Japan and Mexico. Breakpoint sequence analyses showed nonrecurrent deletions in 5/6 families. The deletions varied from 35 Kbp to 80 Kbp in size, but invariably encompassed or interrupted only two genes: TGDS encoding the TDP-glucose 4,6-dehydratase and GPR180 encoding the G protein-coupled receptor 180, also known as intimal thickness-related receptor (ITR). Unlike TGDS which has no known function in muscle cells, GPR180 is involved in the regulation of smooth muscle cell growth. The identification of a null GPR180 mutation segregating over two generations with iridocorneal angle dysgenesis which can be regarded as a MCOR endophenotype is consistent with the view that deletions of this gene, with or without the loss of elements regulating the expression of neighboring genes, are the cause of MCOR.

C12.6

A molecular network surrounding dysregulated H3K9 di-methylation in PRDM5-associated disease

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Introduction: Brittle cornea syndrome (BCS) is an inherited connective tissue disease with a devastating ocular phenotype. Mutations in transcription factors *ZNF469* and *PRDM5* cause BCS types 1 and 2, respectively. PR domain containing 5 (PRDM5) is hypothesised to exert epigenetic effects on histone and DNA methylation, chromatin organisation, and microRNA regulation, however its role in epigenetic regulation is not fully elucidated. PRDM5-related disease offers an *in vivo* opportunity to observe a subset of epigenetic regulatory mechanisms in an inherited eye disease.

Methods and results: We report a retinal vascular phenotype in the eyes of two patients with PRDM5-associated disease, and through mining PRDM5 ChIP-seq data performed in murine MC3T3 cells and our expression microarray data performed on patient fibroblasts suggest a role for *PRDM5* in vasculogenesis. We confirmed *PRDM5* binding at a subset of vasculogenesis-related genes in skin fibroblasts by ChIP-QPCR. We examined PRDM5 interaction partners by pull-down and mass spectrometry and observed diminished interaction of a PRDM5 construct carrying a BCS-associated mutation with repressive complexes, including NuRD complex protein CHD4, and heterochromatin protein 1 binding protein 3 (HP1BP3). We investigated a role for HP1BP3 *in vivo*, identifying reduced HP1BP3 staining in patient retinas, and identifying H3K9 di-methylation as a molecular mechanism for transcriptional repression at a subset of vasculogenesis-related PRDM5 target genes. Western blotting studies on patient fibroblasts further suggested a role for dysregulated H3K9 di-methylation *in vivo.* **Conclusions:** These findings suggest a role for dysregulated H3K9 di-methylation in PRDM5-associated disease.

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C13.1

Human-specific gene evolution and diversity of the chromosome 16p11.2 autism CNV

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Recurrent 600kbp deletions and duplications at 16p11.2 are associated with autism, schizophrenia and extremes of BMI and head circumference. These rearrangements occur via non-allelic homologous recombination (NAHR) between *BOLA2*-containing directly oriented segmental duplications at BP4 (breakpoint 4) and BP5. Illumina sequencing of 2,551 humans, 86 great apes, a Neanderthal and a Denisovan showed that modern humans carry at least one additional copy of *BOLA2* (from 3 to 10 diploid copies) in contrast to apes and archaic hominins. Through Illumina and PacBio sequencing of large-insert clones from orangutan and chimpanzee, we identified three inversions in the human lineage after divergence from orangutan, affecting >1Mbp of sequence and 45 genes, together with the addition of ~1Mbp via segmental duplication. The latter includes a ~102kbp *BOLA2* segment that duplicated ~183kya, the time when *Homo sapiens* emerged as a species.

We sequenced four human haplotypes and discovered multiple ~102kbp tandem duplications in BP4 and BP5, likely leading to different NAHR predisposition. We are currently assaying *BOLA2* copy number and refining breakpoints in >125 patients with a BP4-BP5 deletion or duplication. *BOLA2* is ubiquitously expressed, present in all eukaryotes, and involved in the regulation of iron metabolism. Expression levels in human lymphoblastoids correlate with copy number (r=0.29), expression of genes on 16p13 and 19p13, and of genes encoding mitochondrial and ribosomal proteins.

These findings suggest that a possible advantage linked to the emergence of duplicated genes in the last 200,000 years of human evolution, underlies the predisposition to recurrent rearrangements at 16p11.2 associated with autism.

C13.3

Chromosomal contacts connect loci associated with autism, BMI and head circumference phenotypes

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We identified the cis- and trans-acting long-range chromosomal contacts of five genes located within the 16p11.2 600kb BP4-BP5 copy number variants (CNVs). Its deletion is one of the most frequent known etiologies of autism spectrum disorder (ASD), while this deletion and reciprocal duplication are associated with mirror phenotypes on BMI, head circumference and brain volume. We observed complex chromatin looping between genes located in the proximal 600kb BP4-BP5 and those mapping to the distal 16p11.2 220kb BP2-BP3 region, two loci separated by 650kb, successfully confirmed by reciprocal 4C, FISH, Hi-C and associations between active regulatory regions. Phenotyping of 137 unrelated carriers of distal 16p11.2 220 kb BP2-BP3 deletion and duplication showed that these CNVs are similarly associated with ASD and reciprocal impacts on BMI and HC.

Our results indicate that chromosomal contacts' maps could uncover functionally and clinically related genes. Consistent with this hypothesis, loci chromatin-contacted by our selected viewpoints are enriched for ASD genes (OR=2.15, P=1.65e-08). Furthermore, we uncovered 16p11.2 600kb BP4-BP5 chromatin loops with (i) the 1q21.1 cytoband, whose deletions and duplications were previously linked to micro- and macrocephaly, respectively; (ii) PTEN, mutations of which are associated with a macrocephalic-form of ASD; and (iii) the 2p15 cytoband. We enrolled 35 carriers of 2p15-16.1 deletion and duplication and showed that they similarly display mirror phenotypes on HC and weight.

Finally, we observe that genes differentially expressed in 16p11.2 BP4-BP5 CNV carriers are concomitantly modified in their chromatin interactions, suggesting that disruption of chromatin interplays participates in the observed phenotypes.

C13.2

The impact and activity of mobile elements within the genome

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Mobile genomic element insertions (MEIs) are DNA sequences that can be autonomously copied or moved through the genome, yet their highly repetitive sequence structure makes them difficult to detect and genotype. In addition to being a major evolutionary driver in changing the genomic architecture, MEIs can also directly resulted in pathogenic variation in a number of human diseases by inserting into functionally important regions and disrupting gene function, or indirectly by mediating deletions.

We have developed a method to identify both MEIs as well as gene retrotransposition insertions (GRiPs) in whole genome sequencing data. Using this tool we screened a cohort of 250 trios selected from the Dutch population and whole genome sequenced to a mean depth of 14x. In total 11,680 MEIs and 5 GRiPs were identified. The majority of the MEIs were rare Alu events (83%) with a frequency of less than 1% (56%). Per individual 1,200 MEIs were identified, including exonic insertions predicted to result in loss of function in known disease genes. In addition we identified and validated 5 de novo MEI events and used the trio design to estimate the genotype error rate (0.4%).

Due to developments in NGS it is now possible to perform genome wide identification of both MEI and GRiP events. The discovery of these events in large cohorts provides new insights into the role of genomic variation with positional effects.

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C13.4

Single-cell allele specific expression (ASE) in T21: a novel approach to understand Down syndrome.

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Trisomy 21 is a model disorder of altered gene expression. We have previously used a pair of monozygotic twins discordant for T21 to study the global dysregulation of gene expression, without the noise due to genetic variation among individuals (Nature:508;345-350;2014). The majority of previous studies focused on aneuploidies were conducted on cell populations or tissues. Studies on gene and allelic expression behavior at the single cell level, may reveal important biological insights regarding the cellular impact of aneuploidy and elucidate the fundamental mechanisms of gene dosage. In this study we employed allele specific expression (ASE) using RNAseq from 352 single cell fibroblasts (172 Normal and 180 T21 cells) from the pair of monozygotic twins discordant for T21. A considerable number of heterozygous sites throughout the non-chr21 genome were expressed monoallelically (Normal: 73.2 % monoallelic in 559,134 observations, and T21: 78.8 % monoallelic in 573,670 observations). There was also considerable monoallelic expression for chr21 genes in Normal and surprisingly in T21 cells as well (Normal: 67,2 % monoallelic in 4,985 observations, and T21: 76.07 % monoallelic in 6,723 observations). This metric was used to classify genes on chr21 according to the level of monoallelic expression (9 monoallelic, 29 intermediate, 2 biallelic). We hypothesize that different classes of genes contribute with different mechanisms to the phenotypic variability of Down Syndrome. Furthermore we have made a preliminary observation that genomewide T21 cells showed more monoallelic expression than the normal cells, but more analysis is needed to confirm these results. This study provides a fundamental understanding of the allele specific expression behavior in T21.

C13.5

High incidence of mosaic chromosomal aneuploidies in human cell lines: a quantification of the frequency of the phenomenon

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Maintenance of a balanced euploid genome is a key requisite for the success of all multicellular organisms. Broken chromosomes trigger repair pathways and are known to block or delay mitosis. As a consequence, chromosome segregation is generally thought to accurately transmit intact chromosomes. Estimates of the whole chromosomal error rates derived from karyotyping and FISH studies of cell lines or stimulated white blood cells, range between 2-4x10-2 per cell division. The occurrence of segmental imbalances is estimated to be 5x10-4 per cell division. Sporadic studies of single cell genome wide CNV analysis suggested that the error rate might be higher than currently estimated. To obtain accurate measures of chromosomal error rates, we plated fibroblast and analyzed the genomes of two daughter cells following a single cell division, using array-based approaches. In total 152 cells (76 mitoses) from 5 different normal control fibroblast cell lines were analyzed. At least one imbalance was detected in 23 cells, giving a aneuploidy rate of 5.3-25% (median=15.1%) in the different cell lines tested. In conclusion, the chromosomal stability is more than 100 times lower than current dogma, showing that chromosomal instability is a common place and putting the efficacy of the DNA repair mechanisms and control checkpoints in question.

C13.6

Chromothripsis in healthy individuals affects multiple protein-coding genes and can result in severe congenital abnormalities in offspring

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Chromothripsis represents an extreme class of complex chromosome rearrangements (CCRs) with major effects on chromosomal architecture. Although recent studies have associated chromothripsis with congenital abnormalities, the incidence and pathogenic effects of this phenomenon require further investigation. Here, we analyzed the genomes of three families in which chromothripsis rearrangements were transmitted from a mother to her child. The chromothripsis in the mothers resulted in completely balanced rearrangements involving 8-23 breakpoint junctions across 3-5 chromosomes. Two mothers did not show any phenotypic abnormalities, although 3-13 protein coding genes were affected by breakpoints. Unbalanced but stable transmission of a subset of the derivative chromosomes caused apparently de novo complex copy number changes in two children. This resulted in gene dosage changes, which are likely responsible for their severe congenital phenotypes. In contrast, one child with severe congenital disease harbored all three chromothripsis chromosomes from his healthy mother, but one of the chromosomes acquired de novo rearrangements leading to copy number changes. These results show that the human genome can tolerate extreme reshuffling of chromosomal architecture, including breakage of multiple protein coding genes, without noticeable phenotypic effects. The presence of chromothripsis in healthy individuals affects reproduction and is expected to substantially increase the risk of miscarriages, spontaneous abortions and severe congenital disease.

C14.1

External Quality Assessment of Genetic Counselling: experiences with the first pilot assessment

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Quality assessment has long been associated with laboratory, but not clinical, services. To address this gap, in 2012 the ESHG Genetic Services Quality Committee (GSQC) explored the needs for a European Quality Assessment (EQA) scheme for genetic counselling. All European national societies of human genetics were surveyed. All participating 15 countries expressed a need for an EQA for genetic counselling services.

A proposal for achieving an EQA for genetic counselling was launched at an ESHG satellite symposium in 2013. The working group wrote four case scenarios in the fields of cardiogenetics, oncogenetics, monogenetic disorders and dysmorphology. Each scenario started with a referral letter and consisted of multiple stages, to reflect an episode of clinical care. At each stage more information was given and a number of questions presented. For each question, consensus answers were obtained by the author of the case, a patient organisation and at least two other experts.

A total of 16 genetic centres from 11 countries participated in the pilot EQA

and all answers were reviewed by two assessors per case. The whole process was evaluated, both by the assessors and by the participating centres (post pilot EQA questionnaire). The results highlighted some differences in genetic counselling practice across Europe and indicated that few centres offered psychosocial support. The conclusion was that an EQA for genetic counselling is feasible and highly educational. A second exploratory pilot EQA will be run in 2015 to further improve the process. The EQA process will be demonstrated using one of the educational pilot cases.

C14.2

Hereditary breast and ovarian cancer syndrome: successful, large-scale implementation of a group-based approach to genetic counseling.

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Introduction. In the past two years, there has been a large increase in referrals to our cancer genetics clinic of patients with suspected BRCA1/2-associated hereditary breast and ovarian cancer syndrome (HBOC), probably due to the "Angelina Jolie effect". However, staffing has not increased in proportion, and dealing with the demand has proved challenging. A group-based approach to genetic counseling seemed an appealing way to increase efficiency. Methods. From 9-October-2014, we systematically invited referred patients aged 35-75, with a good performance status and in which assessment for HBOC was warranted, to group-based counseling. It consisted of a 30-minute group session with a genetic counselor and a cancer geneticist, followed by rapid face-to-face standard counseling, and genetic testing when appropriate. Patients completed a form assessing their general understanding of cancer genetics before and after the group session, and a custom-made satisfaction questionnaire before leaving the clinic. Results. As of 23-January-2015, 75 patients with a mean age of 50 had attended group-based counseling. The number of patients seen in a three-hour slot was 6, compared to 4 with traditional individual counseling. Cancer genetics understanding improved by an average of 3/10 points after the group session, and the average satisfaction score was 9.5/10. Conclusion. Large-scale, systematic group-based genetic counseling is feasible in patients with suspected HBOC. This novel approach has allowed us to increase efficiency in the context of an ever-increasing number of referrals, and to maintain waiting times of about a month between the referral and the consultation. Updated data including a greater number of patients will be presented at ESHG 2015.

C14.3

Experiences of systematic genetic testing involving women recently diagnosed with epithelial ovarian cancer: a qualitative study

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Newly available rapid genetic testing (RGT) for BRCA1/2 makes it possible to identify mutation carriers soon after cancer diagnosis. There are benefits for directing women's treatment and providing information for families, but there are ethical concerns about testing this group at this point. These women may be elderly, unwell and may have little or no family history of cancer. Those found to have a mutation then have the task of communicating this sensitive information to their families.

We explore the experiences of women recently diagnosed with epithelial ovarian cancer who have been offered RGT through a project considering the feasibility of integrating RGT into routine oncology services (The GTEOC Study). Using data collected from twelve semi-structured interviews with women that were analysed using Interpretive Phenomenological Analysis, we consider how individuals make sense of genetic testing within their broader cancer and life experiences.

Our results highlight the sensitivity of the timing of discussions about genetic testing during a woman's cancer journey. Women have much emotional work to do as they confront their cancer diagnosis, their own mortality and the impact on their family and others. Though genetic testing is welcomed during this time by the women we interviewed, the burdens and complexities are acknowledged. This foregrounds the necessity for appropriate support for this patient group. Our work contributes to current discussions about the ethical issues arising from the introduction of genetic testing in mainstream medicine.

C14.4

Sharing information with children and young people about adult-onset inherited conditions: Using evidence to improve services for parents and their children

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Background: A growing number of young people are undertaking predictive testing for adult-onset conditions. However, there is a lack of empirical evidence about their information and support needs. Aim: To produce the evidence for, and to develop materials to help parents and professionals in sharing information with young people about serious adult-onset genetic conditions.

Methods: Clinic observations and interviews with young people and parents; an analysis of practitioners' case notes and online web fora for young people; collaborations with patient groups and interviews with genetics professionals. We used two adult-onset inherited disorders as exemplars: Huntington's disease and Familial Hypercholesterolaemia.

Results: In both patient groups the process of genetic testing could be a positive and empowering experience for young people, but for some patients there were gaps in the provision of age-appropriate information and emotional support, resulting in poor experiences. Children and young people could question their own risk earlier than literature suggests. Genetics professionals used a range of strategies to support communication between parents and children, but one third gave limited advice and felt under-skilled in this area. Some parents also felt inadequate time was given to discussion of disclosure strategies. We helped patient groups develop evidence based materials for children and young people.

Conclusions: Practitioners should give serious consideration to parents concerns about disclosure even in treatable inherited conditions, and the provision of age-appropriate information. Facilitating family communication should be a goal of genetic counselling, but training for practitioners is needed.

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C14.5

Attitudes towards returning data to participants in sequencing research

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Genome-wide sequencing in a research setting has the potential to reveal health-related information of personal or clinical utility for the study participant. There is increasing pressure to return research findings to participants that may not be related to the project aims, particularly when these could be used to prevent disease. This cross-sectional, web-based survey investigated the attitudes of 6944 individuals from 75 countries towards returning results from genome research. Participants included four relevant stakeholder groups: 4961 members of the public, 533 genetic health professionals, 843 non-genetic health professionals and 607 genomic researchers who were invited via traditional media, social media and professional email list-serve. Treatability and perceived utility of genomic data were deemed important with 98% of stakeholders personally interested in learning about preventable life-threatening conditions. Participants appeared to assign a value to genomic data, there was a sense of 'if the scientists know it, I'd like to know it too' - 59% were interested in being able to receive their raw sequence data, even though the majority would not do anything with this. 52% of participants felt it was important that genomic researchers kept reanalysing their data and had the ability to update them periodically with new findings; 85% of participants felt that if something serious was discovered they would like this information delivered to them directly by an appropriately trained health professional. This social studies study offers the largest dataset, published to date, of attitudes towards issues surrounding the return of results from sequencing research.

C14.6

Population-based Preconception Carrier Screening: how do potential users view a preconception test for 70 severe autosomal recessive diseases?

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Background
A preconception carrier screening (PCS) test can detect whether a couple has an increased risk of having a child with a monogenetic disease. Next-generation sequencing (NGS) allows the testing of many genes or diseases simultaneously. The University Medical Center Groningen (UMCG) developed an NGS PCS test for couples covering 70 very severe, autosomal recessive diseases simultaneously and screening for all variants. This is the first non-commercial population-based PCS test to be offered to prospective couples in a healthcare setting in Europe. So far, little is known about how potential users view such a population-based PCS test.
Methods
We examined potential users' intentions to undergo the test and preferences regarding who should offer the test. Data was collected in March 2014 by means of an online survey among 500 people belonging to the target population (people aged 18-40, with a partner and living in the Netherlands).
Results
One third of the respondents said they intend to take this test were it to be offered. They prefer this to be offered via their GP and via face-to-face pre-test consultation. Fifty-eight percent is willing to pay for the test, up to a cost of 5,000 euro. Testing for later onset, treatable diseases or even traits (e.g sportivity) is valued less. The UMCG will start an implementation study soon in which the PCS test is supplied via selected GPs in the north of the Netherlands. Our first aim is to measure the uptake, practical feasibility and psychological impact of offering the test.

C15.1
Genome-wide association study of 200,000 individuals identifies 18 genome-wide significant loci and provides biological insight into human cognitive function

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Educational attainment, measured as years of schooling, is commonly used as a proxy for cognitive function. A recent genome wide association study (GWAS) of educational attainment conducted in a discovery sample of 100,000 individuals identified and replicated three genome-wide significant loci. Here, we report preliminary results based on conducted in 200,000 individuals. We replicate the previous three loci and report 15 novel, genome-wide significant loci for educational attainment. A polygenic score composed of 18 single nucleotide polymorphisms, one from each locus, explains ~0.4% of the variance educational attainment. Applying data-driven computational tools, we find that genes in loci that reach nominal significance ($P < 5.0x10-5$) strongly enrich for 11 groups of biological pathways (false discovery rates < 0.05) mostly related to the central nervous system, including dendritic spine morphogenesis ($P=1.2x10-7$), axon guidance ($P=5.8x10-6$) and synapse organization ($P=1.7x10-5$), and show enriched expression in various brain areas, including hippocampus, limbic system, cerebral and entorhinal cortex. We also prioritized genes in associated loci and found that several are known to harbor genes related to intellectual disability (SMARCA2, MAPT), obesity (RBFOX3, SLITRK5), and schizophrenia (GRIN2A) among others. By pointing at specific genes, pathways and brain areas, our work provides novel biological insights into several facets of human cognitive function.

C15.2
Systematic phenotype-based deconvolution of intellectual disability disorders into biologically coherent modules

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Intellectual disability (ID) disorders, due to their frequency and enormous genetic and phenotypic heterogeneity, represent a major unmet challenge in health care and diagnostics. A comprehensive and systematic understanding of ID disorders and their underlying biology is still limited. We established a curated database of 650 currently known ID genes, linked them to various functional datasets and classified them according to ID manifestation and severity and 27 associated core clinical features such as organic or neurological anomalies. Using this integrated resource we show that ID genes are substantially enriched in protein-protein interactions and co-expression, highest in the hippocampus. 86% of ID genes fall into 32 common Gene Ontology based molecular processes: metabolism and nervous system development among the largest, and hedgehog and glutamate signalling among the most enriched groups. Identification of highly enriched functional themes and phenotypes systematically revealed characteristic phenoprofiles of process-defined IDopathies including chromatin- and DNArepairopathies. Strikingly, classification of ID genes according to their associated clinical phenotypes efficiently breaks them down into subsets with significantly elevated biological coherence and allows ID genes to predict each other. Furthermore, we utilised custom-made datasets on ID gene function in Drosophila. Early onset behavioural and specific morphological wing phenotypes were characteristic for ID genes in general, and several fly phenotypes were particularly representative for specific human clinical phenotype classes. Our study and resource provide systematic insights into the molecular and clinical landscape of ID disorders and prove the utility of systematic human and cross-species phenomic analyses in highly heterogeneous genetic disorders.

C15.3
9.6% of mouse gene knockouts show abnormal neuroanatomy: a resource to identify genes and gene networks involved in ID in human

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Intellectual disability (ID) affects 1-3% of the general population. Genetic mutations account for about half of the currently undiagnosed cases, and despite recent successes in identifying some of the mutations responsible, it has been suggested that up to 1,000 further genes remain to be identified. To identify genes involved in brain malformation and potentially associated with ID, we are collaborating with the Sanger Mouse Genetics Project (MGP), allied to the International Mouse Phenotyping Consortium (IMPC), to systematically study the neuroanatomy of the MGP/IMPC knockout mouse strains. We are measuring a standardized set of 78 brain parameters across 22 brain regions to detect a variety of mechanisms that underlie brain malformation disorders, such as aberrant cell proliferation, neuronal migration defects or elevated cell death rates.
So far, we have assessed brain defects in 825 knockout mouse genes. These preliminary data yielded success with the identification of 40 known ID genes including Ap4e1, Cenpj, Chd7, Mcph1, Sc4mol and Ube3b demonstrating the pertinence of our approach. We also discovered 41 other genes including Mta1, Ccdc104, Caprin2 and Dusp3, which when disrupted caused modification of brain structures and thus are good candidate genes for ID.
Our study is the largest screen of brain morphology from the MGP/IMPC. It shows that we can detect abnormalities in about 10% of knockout mouse mutants, and that these translate into human pathology. This offers a complementary resource to human genetic studies.
The project is funded by the French National Research Agency and the Swiss National Science Foundation.

C15.4
Finding new connections in the transcriptional regulation of Lysine-specific demethylase 5C (KDM5C) a disease gene involved in syndromic and non-syndromic XLID

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X-linked Intellectual Disability (XLID) is a group of heterogeneous disorders caused by mutations in genes on the X chromosome. Disease mutations in ~10% of X chromosome genes are implicated in causing XLID disorders in ~50% of known XLID disorders.
Mutations in Lysine-specific demethylase 5C (KDM5C) gene have been reported as an important cause of both syndromic and non-syndromic (XLID)

in males. KDM5C is a chromatin remodelling regulator with histone demethylase activity for di- and trimethylated histone 3 lysine 4 acting as transcriptional repressor during brain development and neuronal maturation. With Regulatory Element-1-Silencing Transcription factor (REST), a critical regulator of the spatio-temporal transition of neural progenitors to neurons, KDM5C co-occupies the promoters of a subset of REST target genes.
We identified a disease path, linking functionally KDM5C to another XLID/Epilepsy gene, encoding the homeotic transcription factor ARX, whose mutations impair severely KDM5C transcript regulation.
Furthermore, we analysed two additional XLID proteins that also bind KDM5C promoter. They are PHD Finger Protein 8 (PHF8), a H3K9 demethylase; and Zinc Finger Protein 711 (ZNF711), a transcriptional factor, which role is almost unknown. We observed that PHF8 and ZNF711, which co-occupy the target promoter, induce cooperatively the KDM5C stimulation. This activity seems to be ARX-independent and we propose that the transcriptional induction by ARX does not synergize with the action of the PHF8/ZNF711 complex. Remarkable, our findings open new perspectives towards the exploitation of rational strategies to treat the growing group of ID and cognition diseases that are caused by chromatin and/or transcriptional defects.

C15.5
HCFC1 is a dosage sensitive transcriptional coregulator of neurodevelopment that influences neural progenitor and neuronal cell function

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Initially we implicated HCFC1 in X-linked intellectual disability (XLID) by a non-coding, regulatory mutation. The single base change abolished binding of the YY1 transcription factor at a highly conserved sequence proximal to the HCFC1 transcription start site resulting in loss of transcriptional repression. We employed ex-vivo models of embryonic neural development to show that this change was likely pathogenic; over-expression of HCFC1 caused cell cycle exit and differentiation of neural progenitor cells (NPCs), and reductions in neurite growth of hippocampal neurons. Intriguingly, missense mutations that result in almost complete loss of HCFC1 have also been reported to cause Cobalamin type X (CblX), a VitaminB12 metabolic disorder with severe neurodevelopmental impairment including intractable epilepsy. We extended our studies to show that in contrast to over-expression, modest reduction (~50%) of Hcfc1 expression promoted the cell cycling of NPCs at the expense of differentiation, and enhanced neurite growth of neurons. We further identified four additional missense variants in HCFC1 that segregate with ID in four families. Three of these variants caused partial loss of function in multiple cell based assays, including complementation of neurite growth. In line with only partial loss of function, metabolic features of CblX were largely absent in affected individuals. Our identification and functional assessment of HCFC1 mutations together with descriptions of a broadened phenotypic spectrum reveals an emerging genotype-phenotype correlation, dependent also on HCFC1 abundance and not just function. Furthermore, we have identified relevant disease mechanisms of ID that converge on the behaviour of cells present during embryonic stages of brain development and Cobalamin metabolism.

C15.6
Clinical and experimental evidence establish a link between *KIF7* and *C5orf42*-related ciliopathies

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Joubert syndrome (JBTS) is a ciliopathy characterized by developmental delay, oculomotor apraxia, breathing abnormalities and a distinctive midhindbrain malformation on brain imaging known as molar tooth sign. It is genetically heterogeneous with >20 causal genes identified to date and its clinical and neuroradiologic findings overlap with other ciliopathies such as acrocallosal syndrome (ACLS). However, craniofacial manifestations of macrocephaly, prominent forehead and hypertelorism have been distinctive features of ACLS among ciliopathies. Here, we evaluated eight families with ACLS-like craniofacial appearance and other ciliopathy features by whole exome or targeted Sanger sequencing which revealed biallelic mutations affecting *KIF7*, the ACLS gene, or *C5orf42*, the JBST gene. Given the known role of KIF7 in primary cilia and Hedgehog signaling, we wondered if there was a functional connection to C5orf42 which's function has remained unknown. Therefore, we also assessed C5orf42 by evaluation of the primary cilia in affected individuals and *in ovo* RNAi silencing in chicken embryos. Consequently, in addition to the clinical overlap between *KIF7* and *C5orf42*-related craniofacial features, we found abnormal primary cilia in the *C5orf42*-patients and evidenced its role in craniofacial development, pathfinding of commissural axons and neural circuit formation in developing chicken embryos.

C16.1
Systematic evaluation of patients with idiopathic short stature using whole exome sequencing

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Shortness of stature is a common medical concern in childhood and has an incidence of 3% in the general population. After excluding defects of the growth hormone pathway and recognizable syndromes the underlying cause remains unknown in approximately 70-80% of patients.
In some of these patients the underlying diagnosis is omitted by the lack of clinical features characteristic for known syndromic forms of short stature. To address this in patients with idiopathic short stature we thoroughly built a study group of more than 500 families with idiopathic short stature. We systematically selected 100 individuals where growth hormone defects, common genetic causes of short stature or copy number variations were excluded and performed whole exome sequencing. Variants were selected unbiased based on all modes of inheritance in agreement with the segregation in the families and their potential effect on protein function using our NGS Variant Analyzer software.
We confirmed mutations in known short stature genes in 11 patients. All these syndromes have been reported to be associated with further clinical issues providing mandatory medical guidance for these patients.
Furthermore, we recognized recessive, dominant and x-linked inherited variants in novel candidate genes involved in epigenetic modification, cell cycle regulation, ubiquitination and protein synthesis.
In conclusion, whole exome sequencing identified the underlying genetic defect in 11% of the patients with idiopathic short stature. As the clinical spectrum of most genetic defects is yet to be explored, an unbiased genetic analysis of patients with idiopathic short stature can establish a diagnosis in these cases.

C16.2
Mutations in the core NHEJ components LIG4 and XRCC4 result in microcephalic primordial dwarfism

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Non-homologous end joining (NHEJ) is a vital cellular process repairing

DNA double strand breaks. Previously, mutations in NHEJ pathway components have predominantly been associated with severe combined immunodeficiency, consistent with the requirement for NHEJ during V(D)J recombination to ensure diversity of the adaptive immune system. In contrast, we recently described biallelic truncating mutations in *LIG4* as a common cause of microcephalic primordial dwarfism (MPD), a disorder of prenatal-onset extreme global growth failure; identifying a genotype-phenotype correlation where the severity of growth failure is related to disruption of the XRCC4 binding domain in LIG4. LIG4 is required for the final ligation step in NHEJ forming a complex with XRCC4 and NHEJ1. Given its candidacy, we have now identified biallelic *XRCC4* mutations in a further five families and provide confirmatory cellular studies establishing *XRCC4* as a disease causing gene. Both patient groups show similar anthropometric measurements with severe microcephaly and short stature as well as similar facial features. However, in contrast to LIG4 patients, pancytopenia leading to bone marrow failure has not been observed in XRCC4 patients and overt immunodeficiency is not apparent on clinical investigation despite reduced junctional diversification. These findings suggest differential developmental requirements for growth and immunity by specific components of the NHEJ pathway. We found *LIG4* and *XRCC4* mutations to be the second most common cause of MPD in a large patient cohort behind mutations in *PCNT* (MOPDII) highlighting the importance of NHEJ, and LIG4-XRCC4 binding in particular, in promoting normal growth.

C16.3
Loss-of-Function Mutations in WDR73 Are Responsible for Microcephaly and Steroid-Resistant Nephrotic Syndrome: Galloway-Mowat Syndrome

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Galloway-Mowat syndrome is a rare autosomal-recessive condition characterized by nephrotic syndrome associated with microcephaly and neurological impairment. Through a combination of autozygosity mapping and whole-exome sequencing, we identified WDR73 as a gene in which mutations cause Galloway-Mowat syndrome in two unrelated families. WDR73 encodes a WD40-repeat-containing protein of unknown function. Here, we show that WDR73 was present in the brain and kidney and was located diffusely in the cytoplasm during interphase but relocalized to spindle poles and astral microtubules during mitosis. Fibroblasts from one affected child and WDR73-depleted podocytes displayed abnormal nuclear morphology, low cell viability, and alterations of the microtubule network. These data suggest that WDR73 plays a crucial role in the maintenance of cell architecture and cell survival. Altogether, WDR73 mutations cause Galloway-Mowat syndrome in a particular subset of individuals presenting with late-onset nephrotic syndrome, postnatal microcephaly, severe intellectual disability, and homogenous brain MRI features. WDR73 is another example of a gene involved in a disease affecting both the kidney glomerulus and the CNS.

C16.4
Mutations in PLK4, encoding a master regulator of centriole biogenesis, and its substrate, TUBGCP6, cause microcephaly, growth failure and retinopathy

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Centrioles are microtubule-based structures that form the core of the centrosome and are essential for ciliogenesis. However, mutations in centriole biogenesis genes have been reported in primary microcephaly and Seckel syndrome, disorders without the hallmark clinical features of ciliopathies. Through linkage analysis and exome sequencing, we have identified mutations in the gene, PLK4, encoding PLK4 kinase, a master regulator of centriole duplication, in individuals with microcephalic primordial dwarfism. PLK4 individuals also demonstrated retinopathy, a phenotypic feature normally associated with cilium dysfunction. Two distantly related individuals are homozygous for a frameshift mutation, where detailed molecular characterization revealed a previously unrecognized alternative isoform which rescued the null allele. An additional mutation was identified in a large consanguineous family, where splicing altered the reading frame of the terminal exon. Using cellular and developmental systems we established that, through reduction in centriole number, PLK4 mutations resulted in aberrant growth and retinal phenotypes. Exome analysis and resequencing also identified multiple individuals with mutations in the substrate of PLK4, TUBGCP6, in individuals with microcephalic primordial dwarfism and additional congenital anomalies, including retinopathy, thereby extending the human phenotypic spectrum associated with centriole dysfunction. Furthermore, we establish that different levels of impaired PLK4 activity result in growth and cilia phenotypes, providing a mechanism by which microcephaly disorders can occur with or without ciliopathic features.

C16.5
Mutations in TUBGCP4 alter microtubule organization via the γ-tubulin ring complex γTuRC in autosomal recessive microcephaly with chorioretinopathy.

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Inherited congenital microcephaly is a clinically and genetically heterogeneous disorder. Autosomal dominant Microcephaly with or without Chorioretinopathy, Lymphoedema, or Mental Retardation (MCLMR) also known as Alzial syndrome has recently been attributed to mutations in KIF11. Autosomal recessive Microcephaly and Chorioretinopathy with or without Mental Retardation, abbreviated as MCMR, is a very rare entity recently described to be associated with TUBGCP6 mutations.
We have identified TUBGCP4 variants in individuals with autosomal recessive microcephaly and chorioretinopathy. Whole exome sequencing performed on one family with two affected siblings and independently on another family with one affected child revealed compound heterozygous mutations in TUBGCP4. Subsequent Sanger sequencing was performed on a panel of individuals with microcephaly and ophthalmic manifestations and one other patient was identified with compound heterozygous mutations in TUBGCP4. TUBGCP4 encodes the γ-tubulin complex protein 4, a component of the γ-tubulin ring complex (γTuRC) known to regulate the nucleation and organization of microtubules. Functional analysis of patient fibroblasts disclosed reduced levels of the γTuRC, altered nucleation and organization of microtubules, abnormal nuclear shape, and aneuploidy. Moreover, zebrafish treated with morpholinos against tubgcp4 were found to have a reduced head volume and eye developmental anomalies with chorioretinal dysplasia.
The identification of biallelic TUBGCP4 mutations described herein, as well as the very recent TUBGCP6 mutations, confirms the existence of autosomal recessive cases described as microcephaly and chorioretinopathy with or without mental retardation and provides evidence for an important role for the γTuRC in brain and eye development.

C16.6
From whole exome sequencing to functional studies in syndromic microcephaly: using zebrafish for variant testing

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Microcephaly (MC) is a clinical condition characterized by abnormal small head circumference for the respective age and sex which closely correlates with reduced brain volume and most of the times with intellectual disability. Many syndromes encompassing MC have been described and although several genes have been recently reported in literature, a massive number of unexplained cases still exist. We have collected and performed whole exome sequencing (WES) on 21 patients displaying syndromic forms of MC and on their parents for trio-based variant filtering. Since most of the index patients were sporadic, the majority of causative variants were suspected to be de novo. However, recessive models have also been taken into account. We were able to identify causative mutation in 10/21 patients. For the remaining cases, we found de novo mutations in 8 novel candidate genes. We used the zebrafish model to test the pathogenicity of candidate variants. The morphant model for one of our main candidates showed a 10% head size reduction, while preliminary mRNA overexpression data unexpectedly showed a slight head area increase. Our cohort was enriched with patients displaying de novo mutations in CASK, responsible for an X-linked syndrome of MC and pontocerebellar hypoplasia. CASK morphants displayed a pronounced head size reduction, while anti-acetylated tubulin staining revealed prominent cerebellar abnormalities. For both genes, rescue experiments and further functional assays are pending.

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C17.1
RNF12 is essential for X-inactivation in female mouse embryonic stem cells, is required for female mouse development, and might be a target for future therapies to treat X-linked disorders in females: evidence from a mouse knockout model

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X-chromosome inactivation (XCI) in females is a crucial mechanism which equalizes X-linked gene-dosage between both sexes. In mice, a first wave of imprinted XCI occurs during cleavage stages of embryonic development, followed by X-chromosome reactivation (XCR) at the pre-implantation blastocyst, and subsequent random XCI (rXCI) in the post-implantation epiblast. rXCI can be simulated in differentiating mouse ES cells. We have previously shown that the X-encoded RNF12 protein act as a dosage-sensitive XCI-activator (PMID:19945382, PMID:21298085, PMID:24613346). When RNF12 becomes up-regulated during differentiation, it targets the pluripotency factor Rex1 for proteasomal degradation (PMID:22596162). As Rex1 is a repressor of the non-coding Xist RNA, which is crucial for initiating chromosome-wide gene-silencing, down-regulation of Rex1 by RNF12 allows female-specific Xist-expression and XCI-initiation in a stochastic manner (PMID:20083102).Here we present the generation and analysis of a novel Rnf12 knockout mouse model, and provide evidence that RNF12 is also crucial for iXCI and rXCI in vivo. Whereas Rnf12-/Y males are viable, Rnf12-/- female mice fail to undergo XCI leading to lethality at post-implantation. Rnf12-/+ animals inheriting the maternal knockout allele are lethal due to silencing of the paternal Rnf12 allele upon iXCI. Rnf12+/- females inheriting the paternal knockout allele are healthy but show an XCI-defect, with adult cells displaying XCR. This peculiar finding, together with our recent results on XCR in human induced pluripotent stem cells (Stem Cell Reports, in press), opens a new area of research which might lead to novel approaches for treating X-linked diseases, such as Rett syndrome, in females.

C17.2
Pattern of X chromosome inactivation across human tissues - insights from population-scale and single-cell RNA sequencing

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Incompleteness and skewing of X chromosome inactivation (XCI) can result in biases in disease susceptibility and presentation between sexes and across individuals but the full extent and heterogeneity of XCI remains unclear. We have comprehensively profiled the landscape, regulation and variability of escape from XCI by deploying several complementary approaches based on high-throughput RNA sequencing.

Using detailed gene expression data from the GTEx consortium, we show that a large majority of previously reported escape genes demonstrate male/female expression differences detectable at population-level. For many of these genes sex-biased expression is present and directionally similar across the 38 tissues studied, a pattern distinct from autosomal sex-biased expression, suggesting XCI is tightly and uniformly regulated across human tissues. Notably, however, escape genes close to an edge of an escape domain (e.g. KAL1) show more tissue heterogeneity and subtle sex-bias.

To complement these observations and assess individual-level variability in escape we have analyzed single cell RNA-seq data across two tissue types, and assessed the allelic imbalance across the X chromosome from deep sequencing of 17 tissues from a female with non-random XCI. These analyses highlight well-known escape genes (e.g. USP9X), replicate novel candidates from the population-scale analyses (e.g. ZRSR2) and confirm variable escape genes (e.g. TIMP1) and elaborate the underlying dynamics. While finding little evidence for tissue-specific escape the analyses demonstrate tissue heterogeneity in expression from the inactive X (e.g. GYG2).

Together these analyses provide a comprehensive view of the landscape of escape from XCI in adult tissues, essential for understanding the impact of this process on sex differences and inter-individual variability.

C17.3
Genome wide DNA promoter methylation: Differences in human subcutaneous vs. omental visceral adipose tissue

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Background: Differences in DNA methylation pattern between subcutaneous adipose tissue (SAT) and omental visceral adipose tissue (OVAT) may help, together with corresponding changes in the mRNA profile, to elucidate variances in metabolic activity. In the present study we examined genome wide promoter methylation and mRNA expression pattern in 80 paired human SAT and OVAT samples including lean as well as obese subjects.

Methods: DNA methylation analysis was performed using methylated DNA immunoprecipitation and subsequent hybridising on Affymetrix Human Promoter 1.0R tilling arrays. Expression profiles were generated using Illunima human HT-12 chips. All genes with at least 30% methylation difference between SAT and OVAT or lean and obese subjects in the same fat depot were taken forward to overlap analysis with corresponding mRNA expression levels.

Results: We identified 35 genes in the lean and 38 in the obese subgroup conferring negative correlation between DNA methylation and mRNA expression comparing SAT and OVAT. 37 genes were found in the SAT and 76 in the OVAT subgroup comparing lean and obese individuals. The gene lists included known candidate genes such as PPARG but also unexpected genes, e.g. BHMT.

Conclusion: To the best of our knowledge, we show the first genome wide epigenetic data set comparing SAT and OVAT in a considerably large cohort. We identified significant differences in genome wide DNA methylation pattern in paired human SAT and OVAT samples from lean and obese subjects with corresponding changes in mRNA levels. This work helped to identify novel interesting genes related to human obesity.

C17.4
Mapping genetic and epigenetic factors influencing human hippocampal gene expression

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Genome-wide association studies have detected multiple loci associated with psychiatric disorders. The majority of these associated variants are observed in noncoding regions and their functional effects are unclear. Novel

methods allow to systematically investigate the regulatory effects of genetic variants by screening the genome for correlations between allelic variants and gene expression (expression Quantitative Trait Loci / eQTLs) or DNA methylation (meQTLs). Several studies have investigated the occurrence of QTLs in human brain tissue; however the overlap of QTLs between these studies is relatively low.

We employed 150 fresh frozen hippocampal biopsy samples derived from surgery of patients with chronic pharmaco-resistant temporal lobe epilepsy and performed genome-wide SNP genotyping, expression and methylation profiling. Data from 115 brain samples was correlated using a linear regression model. To limit the effect of confounding factors, surrogate variables were included.

At a false discovery rate threshold of 5%, 641 RNA transcripts and 19,953 CpGs showed a genetic regulation by common variants in cis. Enrichment testing of our QTL data among the top hits from the public NHGRI GWAS catalogue revealed an overlap for brain disorders.

Overall, we present an integrative functional analysis to explore the effects of common DNA sequence variants on DNA methylation and mRNA expression. In contrast to all published studies, our samples were collected from fresh frozen and not post-mortem brain tissue. Therefore, the identified QTLs provide an extremely valuable resource for functional annotation of SNPs and will help guiding the interpretation of GWAS hits in genetically complex brain disorders.

C17.5

Analysis of monoallelic expression in human individual cells revealed novel imprinting genes.

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Genomic imprinting is defined as the mutually exclusive expression of either the paternally or maternally inherited allele. Imprinted genes are implicated in the etiology of rare syndromes and have been associated with common diseases such as diabetes and cancer. We aim to identify novel imprinting genes using a Single-Cell (SC) RNA sequencing approach. The detection of average to low expressed genes allows a more comprehensive profiling of the allelic imbalance of each gene as previously reported (Borel et al, AJHG, 2015). From the proband of a family trio, 380 individual fibroblasts were RNA sequenced and more than 770'000 heterozygous SNVs were identified by WGS (25x). For each gene, we analysed the allelic specific expression using an in-house pipeline. We modeled the likelihood of a gene to be monoallelically expressed with a beta-binomial distribution and evaluated the significance of the aggregate monoallelic ratio (reads sum of the most frequent allele per site / total reads) with the log-likelihood test. Genes presenting with a significant (adjusted p-value <0.01) aggregate monoallelic ratio between 0.9-1 were retained. We were able to detect 634 putative imprinted genes and 16 known imprinted genes. We validated 118 of these genes in 165 SC fibroblasts from an independent individual; in addition, 19 were imprinted in 48 SC lymphoblasts from the same individual. A SC transcriptome analysis provides an unprecedented opportunity to discover the full repertoire of imprinted genes per cell type, and will contribute to the understanding of the molecular pathophysiology of genetic disorders. C.B. and F.S. contributed equally.

C17.6

Novel method reveals a large number of expression quantitative trait loci (eQTLs) influencing transcript levels in a Parent-of-origin fashion

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We investigate expression quantitative trait loci (eQTLs) and search for SNPs that have a parent-of-origin effect (POE) with respect to transcript levels in cis. We applied our recently published algorithm [Hoggart et al 2014] to tease out SNPs where the contribution of the maternal allele to the nearby gene expression is different to that of the paternal allele. In the case where each parent's allele has a different effect, the variance in the expression levels within the heterozygous group will be larger than the variance within either homozygous group. We used genome-wide genotyping and gene-expression data from the EGCUT and Groningen cohorts and ran the quicktest software to estimate the difference in effects.

Controlling the type I error at 5%, we selected 4,162 of the 59 million SNP-gene pairs, where we could reject the null hypothesis of no difference between paternal and maternal effects. We applied conservative pruning (based on LD) to end up with 495 candidate lead SNP-gene expression pairs, where the parents' effects are expected to be different. By combining the marginal effects from [Westra et al 2013] and our observed POE estimates for the differences between maternal and paternal effects we found that 135 of these 495 candidates exhibit classical POE, i.e. where the effect from one parent is exactly zero.

These 135 candidates are currently being validated in ~700 trios from the Framingham study to confirm that the expression levels differ between heterozygous individuals that inherited the two alleles from different parents.

C18.1

Disassembly of MINOS complex by *CHCHD10* mutations promotes loss of mitochondrial cristae with defects in mitochondrial genome maintenance and apoptosis

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Recently, we described *CHCHD10* as a novel gene responsible for mtDNA instability disorder by studying a large family with a late-onset phenotype including motor neuron disease, cognitive decline looking like frontotemporal dementia (FTD), cerebellar ataxia and mitochondrial myopathy with accumulation of multiple mtDNA deletions. Subsequently, other groups and ourselves, identified other *CHCHD10* mutations in several cohorts with frontotemporal dementia-amyotrophic lateral sclerosis (FTD-ALS) or with pure familial or sporadic ALS or late-onset spinal motor neuropathy (SMAJ).

Here, we show that CHCHD10 is a component of the mitochondrial inner membrane organizing system (MINOS) complex. The expression of *CHCHD10* mutant allele leads to MINOS complex disassembly and loss of mitochondrial cristae in patient fibroblasts. The abnormalities of the inner membrane are responsible for nucleoid disorganization leading to defect in mtDNA repair after oxidative stress, which explains the multiple mtDNA deletions found in patient muscles. Interestingly, the expression of *CHCHD10* mutant alleles inhibits apoptosis by preventing cytochrome *c* release. This result supports previous studies suggesting that, in some ALS models, motor neuron death can occur *via* caspase-independent apoptotic mechanism.

In conclusion, we show for the first time that mutations in a gene encoding a MINOS component are responsible for human disorder. Dissecting the cellular pathways disrupted by the expression of *CHCHD10* mutant alleles represents, therefore, a golden opportunity to gain powerful insight into the sequence of events that link mitochondrial dysfunction with neurodegenerative disorders.

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C18.2

COQ4 mutations cause a broad spectrum of mitochondrial disorders associated with CoQ10 deficiency

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Primary Coenzyme Q10 (CoQ10) deficiencies are rare, clinically heterogeneous disorders caused by recessive mutations in several genes encoding proteins involved in CoQ10 biosynthesis. CoQ10, a lipoidal quinone, is an essential component of the electron transport chain (ETC), shuttling electrons from complex I/II to complex III. By whole exome sequencing we identified five individuals carrying biallelic mutations in COQ4. The precise function of human COQ4 is not known, but it seems to play a structural role

in stabilizing a multiheteromeric complex, which contains most of CoQ10 biosynthetic enzymes. The clinical phenotypes of the five subjects varied widely, but four had a prenatal or perinatal onset with early fatal outcome. Two unrelated individuals presented with severe hypotonia, bradycardia, respiratory insufficiency and heart failure; two sisters showed antenatal cerebellar hypoplasia, neonatal respiratory distress syndrome, and epileptic encephalopathy. The fifth subject had early-onset but slowly progressive clinical course, dominated by neurological deterioration with hardly any involvement of other organs. CoQ10 amount was reduced in all available specimens from mutant subjects, often associated with decrease of CoQ10-dependent ETC complex activities and reduced oxygen consumption rate in cultured cells. The pathogenic role of all identified mutations was experimentally validated in a recombinant yeast model: oxidative growth, strongly impaired in strains lacking COQ4, was corrected by expressing a human wild-type COQ4 cDNA but failed to be corrected by expressing COQ4 cDNAs with any of the nucleotide variants identified in affected subjects. COQ4 mutations are responsible for early-onset mitochondrial diseases with heterogeneous clinical presentations associated with CoQ10 deficiency.

C18.3

MCT1 deficiency impairs ketone utilization and causes profound ketoacidosis upon catabolic stress

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Ketoacidosis is a potentially lethal condition caused by an imbalance between ketone body production and ketone body utilization. Ketone bodies are mainly produced in the liver and used as an energy source in extrahepatic tissues. Currently only two genetic defects in ketone body metabolism are known, whereas most patients with recurrent ketoacidosis remained unresolved.

We performed targeted exome sequencing of homozygous genomic regions in a patient from consanguineous parents with recurrent massive ketoacidosis. We sequenced the main candidate gene and related genes in a cohort of 96 patients suspected of a ketolysis defect, followed by functional, biochemical and clinical characterization.

We identified a homozygous frameshift mutation in the monocarboxylate transporter 1 (MCT1/SLC16A1), a known transporter of monocarboxylates including lactate and ketones. Subsequent analysis of MCT1 and related genes in our cohort yielded 6 additional truncating mutations and 1 missense mutation in MCT1. We identified both homozygous and heterozygous inactivating mutations, correlating with the depth of ketoacidosis. Immunoblot analyses revealed reduced and absent levels of MCT1 in heterozygous and homozygous patients, respectively. A lactate transport assay in erythrocytes showed absence of transport in homozygous patients and reduced transport in heterozygotes.

We show that MCT1 deficiency is a novel cause of profound ketoacidosis. Our results indicate that MCT1 is pivotal for import of ketones in extrahepatic tissues. Contrary to the current concept of freely diffusing ketone bodies, our study shows that during catabolic stress, facilitated transport of ketones by MCT1 is essential to allow adequate ketone utilization and maintain acid-base balance.

C18.4

Rare non-synonymous variations in the human ferroportin iron transporter gene (haemochromatosis type 4): the quest for causal mutations

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Haemochromatosis type 4 is a rare form of primary iron overload transmitted as an autosomal dominant trait caused by mutations in the gene encoding the iron transport protein ferroportin 1 (*SLC40A1*). *SLC40A1* mutations fall into two functional categories (loss- versus gain-of-function) underlying two distinct clinical entities (haemochromatosis type 4A-B). However, the vast majority of *SLC40A1* mutations are rare missense variations, with only a few showing strong evidence of causality. The present study reports the results of an integrated approach collecting genetic and phenotypic data from 44 suspected haemochromatosis type 4 patients, with comprehensive structural (3D model) and functional annotations.[1,2] Causality was demonstrated for 10 missense variants, showing a clear dichotomy between the two haemochromatosis type 4 subtypes. Two subgroups of loss-of-function mutations were distinguished: one impairing cell surface expression and one altering only iron egress. A new gain-of-function mutation was identified, and the degradation of ferroportin on hepcidin binding was shown to probably depend on the integrity of a large extracellular loop outside of the hepcidin-binding domain. Eight further missense variations, on the other hand, were shown to have no discernible effects at either protein or RNA level; these were found in apparently isolated patients and were associated with a less severe phenotype. The present findings illustrate the importance of combining in-silico and biochemical approaches to fully distinguish pathogenic *SLC40A1* mutations from benign variants. This has profound implications for patient management.

[1] Le Gac G *et al.* Hum Mut. 2013;34:1371-80

[2] Callebaut I *et al.* Hum Mol Genet. 2014;23:4479-90.

C18.5

Companion diagnostics by comprehensive targeted NGS with evidence for a threshold model in a cohort of 605 patients with atypical haemolytic uremic syndrome and hereditary glomerulopathies

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Genetic defects are responsible for the majority of primary renal diseases leading to end-stage renal disease. The risk of recurrence after transplantation depends on the genotype. Genetic testing becomes increasingly important for proper clinical management and therapeutic and prognostic issues, but there is a need for novel comprehensive, time- and cost-efficient strategies. We analysed 605 unrelated patients by a customized NGS-panel of 347 genes for atypical haemolytic uremic syndrome (aHUS) and hereditary glomerular disorders (nephrotic syndrome, FSGS, Alport syndrome, MPGN, C3 glomerulopathies) which often show clinical and genetic overlap. The alternative complement pathway is typically overactivated in aHUS and C3 glomerulopathies, but genes implicated in coagulation and haemostasis play a pivotal role too. Secondary triggers (e. g., hypertension, pregnancy, transplantation, infection) and predisposing polymorphisms lower the threshold for disease onset. Our study represents the by far largest cohort analysed by comprehensive genetic testing. We present new disease genes and demonstrate that variations in more than one gene contribute to the phenotype with variable expressivity and incomplete penetrance. We show that detailed information on the genotype is crucial for decisions on transplantation, recurrence risk and treatment such as when and how long an expensive drug like the monoclonal antibody eculizumab should be given. The diseases discussed represent an interesting model that may help to explain basic genetic principles not confined to renal disorders and of relevance for all geneticists. We demonstrate that genetic testing assists in the decision-making process to treat patients adequately while handling public resources responsibly.

C18.6

Common and rare variants associated with kidney stones and biochemical traits

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To search for novel sequence variants that confer risk of kidney stones, we conducted a genome-wide association study using 28.3 million sequence variants identified through whole genome sequencing of 2,636 Icelanders and subsequent imputation into 5,419 kidney stone cases, including 2,172 cases with a history of recurrent kidney stones, and 279,870 controls. In addition to replicating previous findings in an Asian population at *SLC34A1* (rs12654812[A] ,OR = 1.18, $P = 5.7\times10^{-11}$) we identified sequence variants associating with kidney stones at *ALPL* (rs1256328[T], odds ratio (OR) = 1.21, $P = 5.8\times10^{-10}$) as well as a suggestive association at *CASR* (rs7627468[A], OR = 1.16, $P = 2.0\times10^{-8}$). We specifically focused our analysis on coding sequence variants in 63 genes with preferential gene expression in the kidney. The strongest associations observed were of two rare missense variants SLC34A1 p.Tyr489Cys (OR = 2.38, $P = 2.8\times10^{-5}$) and TRPV5 p.Leu530Arg (OR = 3.62, $P = 4.1\times10^{-5}$) for recurrent kidney stones. We further tested the sequence variants identified in this study for association with biochemical traits involved in calcium-phosphate and purine metabolism, kidney function, acid-base and ion homeostasis in a large population set. The results demonstrate the role of sequence variants at genes involved in calcium-phosphate homeostasis in the pathophysiology of kidney stones in humans.

C19.1

Large-scale, high-throughput testing of cancer predisposition genes using the TruSight Cancer panel

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Genetic testing of cancer predisposition genes is a major activity of Clinical Genetics, but gene testing in most countries remains very restricted with respect to the number of genes and number of people that can access testing. In collaboration with Illumina we developed an NGS pulldown panel called TruSight Cancer Panel (TSCP), which targets 97 cancer predisposition genes including *BRCA1* and *BRCA2*. TSCP requires only 50ng of input DNA and we multiplex to sequence 96 samples at a time on a HiSeq2500, generating median coverage of 500X. We have developed and validated a bespoke analytical pipeline that detects small and large variants, which runs overnight with no bioinformatic input.

To date we have processed >5000 individuals with TSCP and these data will be presented. Furthermore, TSCP has now been implemented in our accredited clinical testing laboratory, TGLclinical, as the test for *BRCA1* and *BRCA2* mutations, with all mutations validated by Sanger or MLPA as appropriate. 376 clinical reports using TSCP have been issued, including 42 with pathogenic mutations. Using our laboratory and analytical TSCP pipeline one person can process 96 samples from DNA to variant calling in eight working days. The average turnaround time from sample receipt to report issue is now 27 days. Expansion of clinical testing to other genes on the panel is underway. These data provide multiple insights into integration of NGS panel analysis in the clinical setting. This work was undertaken as part of the MCG programme (www.mcgprogramme.com), funded by the Wellcome Trust Grant 098518/Z/12/Z.

C19.2

Increasing accessibility and affordability of genetic testing through targeted clinical exome sequencing

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900 patients have been sequenced for 4813 clinically relevant genes using the Illumina TruSight One sequencing assay. This test has replaced numerous alternative diagnostic sequencing tests, providing a single laboratory workflow and financial savings.

Data is analysed for a panel of genes appropriate for the clinical presentation. To date 190 virtual gene panels have been developed, incorporating 1085 genes, averaging 7.9 genes per panel. Almost 1/3 of panels established (60) are single genes, requested for patients with homogeneous clinical presentations. Single gene analysis yields a higher mutation detection rate (40%) than panel testing (27%), however we will present data showing that panel testing would increase the diagnostic yield further if applied instead of single gene analysis.

Gene panels are established on demand. As long as the required gene(s) are captured and yield good sequencing coverage, a diagnostic test, which may be unavailable or unaffordable by alternative methodology, can be offered almost immediately. For example, we were able to offer rapid testing for a couple presenting in pregnancy, after having a child with a tentative diagnosis of Schwartz-Jampel syndrome. Sequence data was filtered for skeletal dysplasia and distal arthrogryposis gene panels. A biparentally inherited homozygous pathogenic HSPG2 variant was detected in the affected individual, confirming the diagnosis and enabling prenatal testing within 3 weeks of receiving the referral.

This "clinical exome" sequencing approach offers flexible testing at ~25% cost reduction over alternative tests, significantly increasing accessibility to genetic testing for patients with a huge range of genetic disorders.

C19.3

The RD-Connect platform includes the first 360 analysed exomes linked to phenotypic data and integrates user-friendly tools for rare disease variant prioritization

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Around 300 million people worldwide are estimated to suffer from one of the 6000+ known rare diseases. However, rare disease research faces particular challenges because patient populations, clinical expertise, and research communities are small in number and highly fragmented. To overcome these challenges the EU FP7-funded RD-Connect project, in collaboration with Neuromics and EURenOmics, is building a platform to harmonise and securely integrate clinical data with biosample and -omics data. The genomics side of the platform already includes over 360 NGS exomes linked to detailed phenotypes stored in PhenoTips using the Human Phenotype Ontology. Exomes were processed with v1 of the RD-Connect standard analysis pipeline for genomics, which exceeds 99% precision and sensitivity when compared to the NIST reference set of calls for NA12878. The platform runs on a Hadoop cluster and uses technologies such as ElasticSearch, Postgres, Scala and Angular.js, making it highly configurable and efficient. The exomes can be combined in a very flexible manner and variants can be filtered and prioritized through the user-friendly front-end using the most common quality, genomic location, effect, pathogenicity and population frequency annotations, including CADD and ExAC. Moreover, additional tools can be

integrated at the database level or at the interface through API queries. To date, UMD Predictor, DiseaseCard, Alamut Functional Annotation (ALFA) and gene-disease relationships in nanopublication format have been integrated. The project aims to publicly release the first version of the platform during 2015 for authorized users and will gladly accept submissions from other projects in the future.

C19.4
Copy Number Analysis using Exon-level aCGH and Exome Sequencing in over 3,000 Parent-Offspring Trios from the Deciphering Developmental Disorders Project

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The Deciphering Developmental Disorders (DDD) project is a family based study focused on rare genetic variants in patients with undiagnosed developmental delay. Copy number (CNV) discovery in the DDD project is achieved using a combination of exon-resolution array comparative genomic hybridization (exon-CGH) and whole exome next generation sequencing (WES) platforms. We have developed a new algorithm - CIFER (CNV Inheritance From Exome Resources) that uses trio WES for the validation and inheritance classification of CNVs detected using exon-CGH in probands. We screen the genomes of over 3,000 parent-offspring trios for potentially pathogenic CNVs to elucidate the genetic basis for numerous patient phenotypes from the DDD project. The inheritance patterns for CNVs across the patient cohort are explored and compared to current CNV mutation rate estimates from normal controls. An investigation into the potential for using WES as the sole source for CNV discovery comparing against and using exon-CGH as the gold standard is undertaken. By applying an automated CNV filtering pipeline we present the overall characteristics of CNVs predicted to have broad clinical relevance in approximately 10% of proband samples. We provide examples of recurrent CNVs in single known DD genes across multiple patients with similar phenotypes. Additionally by using association based testing and gene haploinsufficiency predictions we present initial findings into suggestive novel DD genes based on CNV frequency rates, inheritance patterns and patient phenotype similarities.

C19.5
Small exonic CNVs as causes of primary immunodeficiencies

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Primary immunodeficiencies (PIDD) constitute a heterogeneous group of genetic diseases affecting the immune system. Mutations in more than 300 genes are known to cause PIDD and the subtype symptoms can be overlapping. Both disease severity, which can range from mild to life-threatening, and treatment options depend on the genetic aetiology. We examined the utility of whole exome sequencing (WES) to detect disease-causing single nucleotide variants (SNVs) and copy number variations (CNVs) in PIDD patients. Patients were recruited from Texas Children's Hospital (Houston, US) and Oslo University Hospital (Norway). As of February 2015, patients with extensive immunological and genetic testing from 258 families have been WES tested. PIDD-causing SNVs were identified in 100 of the families. A computational CNV prediction pipeline was applied to the exome data to identify PIDD-causing CNVs. The predicted PIDD-causing CNVs were validated using a custom array CGH (aCGH) platform with exon-level resolution. The same aCGH was also applied to a selection of the families with negative WES. PIDD-causing CNVs were identified in 10 families, involving MAGT1, SMARCAL1, DOCK8, IL7R, MYB, PGM3, DKC1, and FANCA. Five of the CNVs spanned between 1-4 exons and would not have been detected by traditional diagnostic aCGH, with a typical resolution of 50 kb. This study shows that exome sequencing is an efficient method to establish a genetic diagnosis for PIDD patients and that small exonic CNVs might be a neglected cause of PIDD.

C19.6
Whole genome sequencing as a clinical diagnostic tool for heterogeneous Mendelian disease

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Background: Whole genome sequencing (WGS) enables the analysis of genome-wide genetic variation that can range from single nucleotide alterations to large structural genomic variants. WGS has become more affordable and presents dramatic opportunities for a potential shift in diagnostic services for genetic disease. This is exemplified by the recent commission of the 100,000 Genomes Project in England and the 'precision medicine' programme in the USA. However, diagnostic WGS pipelines are yet to be validated as a clinical tool in the context of heterogeneous genetic disease.
Methods: We surveyed the diagnostic yield of an accredited, conventional next-generation sequencing (NGS) diagnostic testing method for 537 patients with genetically and clinically heterogeneous single gene disorders (specifically, inherited-retinal-disease). A subset of 47 patients also underwent WGS, and we compare the diagnostic yield of a WGS pipeline to that achieved by the conventional NGS diagnostic test.
Findings: The WGS pipeline achieved similar sensitivity and specificity rates to the targeted NGS diagnostic testing method. Importantly, the WGS pipeline detected pathogenic variants that were not identified by current NGS diagnostics and, amongst the 47 patients, facilitated an 85% increase in diagnostic yield. If applied to the 537 patients surveyed, weighted estimates suggest that the WGS pipeline could provide a 27% increase in diagnostic yield.
Interpretation: We demonstrate the capability of a WGS pipeline to detect pathogenic genetic variants missed by current methodologies, and to identify essential modifications for current and future molecular diagnostic practice. We establish the potential utility of diagnostic WGS pipelines for clinically and genetically heterogeneous Mendelian disorders.

C20.1
Ethical and legal challenges of genomic cloud computing

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Genomic researchers are increasingly utilizing vast computer infrastructure and advanced software tools to store and perform comprehensive analyses of genomic data sets. Cloud computing is harnessed both to integrate data from multiple sources and to analyze data to solve biomedical problems. Cloud computing offers multiple advantages, including lower costs and the enablement of greater international collaboration and research efficiency. Yet, it also brings a number of new ethical and policy issues, particularly those concerning the protection of personal health data in an environment of transnational, multidirectional data flow. Because cloud computing affects multiple stakeholders situated across multiple jurisdictions, careful legal and ethical analysis is warranted. In this presentation, we will characterize and analyze the specific legal and ethical challenges emerging from cloud-based genome analysis based on our analysis of publicly available cloud service providers' Terms of Service. These challenges include: data control; data security and privacy; international data transfer; and, accountability. Additionally, in conjunction with the collaborative work of P3G-IPAC (Public Population Project in Genomics and Society - International Policy interoperability and data Access Clearinghouse) and the Global Alliance for Genomics and Health, we will present an international policy for cloud-based genomic research and clinical practice that sets an innovative but effective and ethically responsible approach to conducting genomic medical research in the cloud. Such a policy will robustly protect patient privacy and medical confidentiality, and also facilitate secure access to genomic and clinical data--even when up in the clouds.

C20.2

In utero treatment of Down syndrome - proceed with care

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Non-invasive prenatal testing may well create an opportunity for *in utero* treatment of Down syndrome (IUTDS) by means of compounds improving neurogenesis (1). While research is still in its infancy, experts involved rightly stress the importance of an anticipatory ethical debate. Clearly, IUTDS would raise both conceptual and a cluster of normative issues, linked with research ethics, the ethics of prenatal screening and responsible parenthood.

First, the suggested conceptualization of such treatment as 'fetal personalized medicine' is debatable as this seems to presume a (rightly) contested view regarding the status of the fetus (as a 'person'). Second, future IUTDS will, obviously, be experimental for a longer period. What about the proportionality of possible harms and benefits, taking account of the fact that DS children with a somewhat higher IQ are not necessarily happier? Which criteria should be used to measure success? How to guarantee well-considered decisions of women who may prematurely consider such IUTDS to be evidence-based? Third, (how) would IUTDS change the ethics of NIPT for DS? While IUTDS could circumvent traditional 'pro life' criticism, objections may still come from 'disability rights' criticists - arguing that IUTDS reflects a problematic 'normalization' of people with mental retardation - and from feminists arguing that women may be pressurized to refrain from terminating affected pregnancies, undermining their reproductive rights.

This exploratory ethical analysis will scrutinize the main moral issues and contribute to agenda-setting for further debate

1. Bianchi D. From prenatal genomic diagnosis to fetal personalized medicine: progress and challenges. Nature Medicine 2012;18(7):1-11.

C20.3

Should children's carrier results be reported following diagnostic WES/WGS?

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The ACMG recommend that if children undergo whole genome/exome sequencing (WGS/WES) for diagnostic purposes, results for their predisposition to 24 genetic conditions, predominantly autosomal dominant familial cancer and vascular syndromes, should be reported to clinicians for discussion with the family. They suggest the possible negative psychological impacts from identifying children's genetic risks and the loss of their future autonomy, are outweighed by the potential benefits, both for the health of the child and also their parents.

Yet ACMG do not recommend disclosure of children's carrier status to families, despite the potential benefits to the child, due to the possible health implications for carriers of some X-linked conditions, such as Duchenne Muscular Dystrophy (DMD), and their parents, who may benefit from reproductive choices.

This paper explores the ethical implications of reporting incidental carrier results from diagnostic WGS/WES. We draw on data from qualitative interviews with 17 genetic health professionals and 33 parents of children with genetic conditions (cystic fibrosis, DMD and haemophilia), exploring the practices, views and experiences of carrier testing in children to inform this discussion. We consider these parents' reactions to learning their child's carrier status and their intentions to communicate carrier information to their children, and how this might impact on their abilities to manage incidental findings from diagnostic WES/WGS.

Finally, we draw conclusions about the appropriateness of conveying incidental carrier information from WGS/WES to families in contrast to the recommendations of international guidelines against performing carrier testing in siblings of children with genetic conditions.

C20.4

Informed consent for whole exome sequencing in pediatric disease diagnostics: parental decision-making processes, their ethical relevance and implications for policy development

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Introduction:
The empirical body of evidence on parents' decision-making processes for whole exome sequencing (WES) and return of unsolicited findings (UFs) in pediatric disease diagnostics is limited. Yet understanding these processes and the differences between them is needed for developing morally responsible policy.
Method:
Twenty semi-structured interviews were conducted with parents of children ages <1 – 17, after consenting to WES (trio-analyses), but prior to feedback of results.
Results:
Parental preferences for return of UFs, reasoning patterns, and difficulties experienced in decision-making diverged. Context-specific factors of persons' situations, such as uncertainty about whether their child will develop into an autonomous adult capable of making future disclosure decisions, and differences in persons' moral starting points clarify this divergence. Parents valued being given choices over most outcome categories of UFs for various reasons. One reason was that decisional discretion necessitates contemplation of the possible future consequences of hearing, or not hearing, certain results – thereby ensuring parents' confidence of having made a well-informed decision for WES. A need was sometimes also expressed by parents to understand the moral argumentation behind the center's policy choices regarding opt-ins and opt-outs for certain UFs, since seeing different arguments can help one weigh alternatives in a systematic manner, thus, adding to one's confidence about having decided responsibly.
Conclusion:
These insights inform ethical theorizing on how policy for WES and UFs should be structured in order to ensure persons' well-informed decision-making.
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C20.5

A Human Rights Approach to International Data Sharing?

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Human rights are engrained in international law. They offer many advantages to translational and transnational genome science centred on data sharing. First, their universalizing force can overcome site-specific factors that drive a wedge between research initiative/funder data sharing policies and harmonization. Second, because human rights have both political and legal dimensions, they reach beyond the moral appeals of bioethics and can provide a more robust governance framework for the regulation of genomics research. If health care becomes a primary location for collecting the phenotypic and genetic data needed to create learning systems for research and clinical care, we need to reinforce the self-regulatory codes of ethics of genomic researchers, and clinicians with legally recognized human rights, that is, a *co*-regulatory system. Third, human rights belong to groups as well as individuals (creating a reciprocity between the individual and public level) and reach beyond classic negative duties (i.e. forbidding State actors from interfering with the rights of individuals), to positive, more progressive duties, thereby urging action by governments (and ideally, industry, funders, and researchers) to share the data, technologies and knowledge that are the fruits of our science to achieve a goal desired by all, such as health. Fourth, human rights can foster responsible protection in three critical areas: privacy; anti-discrimination, and procedural fairness. This presentation will use the *Framework for Responsible Sharing of Genomic and Health-Related Data* of the Global Alliance for Genomics and Health as a case study for harnessing human rights as mobilizing force for international data sharing.

C20.6

What's in it for me? A critical analysis of the notion of personal utility in genomic testing

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Observers have suggested that in the ethical evaluation of genomic tests, the criterion of clinical utility should be replaced or complemented with the cri-

terion of personal utility. These suggestions are commonly made from a liberalist vantage point, in which it is held that patients or consumers should be free to define the value of genomic tests for themselves and to access genomic tests - through the healthcare system or through commercial channels - that may lack clinical utility, strictly defined. A paradigmatic example is ApoE testing: while there are limited therapeutic options for Alzheimer's disease (i.e. clinical utility), many people prefer knowing their genetic risk to make life choices or to psychologically prepare for possible scenarios. Further, genomic tests for non-medical traits (e.g. ancestry, professional or sports-related testing) can be interesting or fun and thus have personal value for consumers. But does a 'consumer value' imply that there is personal utility in a genomic test? I will argue that genomic tests that lack predictive ability cannot have personal utility. Further, I will argue - descriptively and normatively - that the notion of personal utility should be demarcated narrowly in order for it to have a role in the ethical evaluation of genomic tests. I will set forth the conditions sine qua non of personal utility in genomic tests, including clinical validity and reasonable potential use. Often, the notion of personal utility is used to ethically justify genomic testing offers to the public; such justifications, I will point out, are flawed.

C21.1

Recurrent de novo p.Arg83Cys mutations in the acetyl CoA binding site of NAA10 are associated with atypical Cornelia de Lange syndrome

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Cornelia de Lange syndrome (CdLS) is a variable multisystem disorder with a broad phenotypic spectrum. Most typically-affected individuals carry *de novo* heterozygous loss-of-function mutations in *NIPBL*. Mutations in other components of the sister chromatid cohesion system, *SMC1A*, *HDAC8*, *SMC3* and *RAD21*, result in phenotypes that overlap with CdLS but which can be highly atypical. In the present study, a recurrent *de novo* mutation was identified in the X-linked gene, *NAA10* (c.247C>T [p.Arg83Cys]) in four unrelated female individuals through trio-based exome sequencing and re-sequencing. A fifth female was found to carry a *de novo* mutation affecting the adjacent codon (p.Arg82Gln). Three of these cases have a CdLS-like appearance, with the growth and dysmorphic features in all individuals being variable. Analysis of X-chromosome inactivation revealed complete skewing of X-inactivation in 3/5 cases and borderline skewing in the remaining two. *In vivo* analysis of a patient fibroblast cell line, only expressing the mutant allele, showed no obvious defects in cell division. Molecular modelling suggests that the p.Arg83Cys conversion is likely to alter binding of NAA10 to acetyl CoA. A significant reduction in auto-acetylation of NAA10 was observed in the presence of the p.Arg83Cys mutation *in vitro*. Furthermore, mouse embryonic fibroblast cell lines derived from CRISPR-induced p.Arg83Cys knock-in mouse embryos are being analysed for further characterisation of this mutation in relation to cohesin function. A detailed clinical comparison to published cases of *NAA10* mutations will also be presented. Our results identify recurrent *de novo* mutations in *NAA10* as a possible cause of CdLS-overlapping phenotypes.

C21.2

STAG1 haploinsufficiency is responsible for a new cohesinopathy with intellectual disability and characteristic facial features in four unrelated individuals

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Cohesinopathies are rare disorders arising from a dysfunction in the cohesin pathway, which enables chromosome segregation and proper cell division. So far, seven genes from this pathway have been reported in human disease. Five of them are involved in autosomal dominant (NIPBL, SMC3, RAD21) or X-linked (SMC1A and HDAC8) Cornelia de Lange syndrome, and two of them in autosomal recessive Roberts (ESCO2) and Warsaw Breakage syndromes (DDX11). All are severe neurodevelopmental conditions characterized by intellectual disability, growth retardation, microcephaly, limb defects and facial dysmorphism.

STAG1 belongs to the STAG subunit of the core cohesin complex, which, along with three other subunits, mediates cohesion between sister chromatids. Here, we report an international series of four unrelated individuals, 3 males and 1 female, aged 8 to 33 years, referred for moderate to severe intellectual disability that could be attributed to STAG1 haploinsufficiency. Three had history of intrauterine growth retardation, two with prenatal-onset microcephaly, one with post-natal growth retardation. One individual had epilepsy, two had autistic features. The four patients shared common facial features, with widely spaced incisors, thin eyebrows, and high nasal bridge. The mechanism for haploinsufficiency was a small deletion encompassing STAG1 diagnosed by array-CGH in two individuals, an intragenic deletion in STAG1 found by whole genome analysis in one individual, and a missense heterozygous mutation (c.641A>G) revealed by exome analysis in the last patient. All the variants were shown to be de novo. This series provides evidence that STAG1 haploinsufficiency leads to a new cohesinopathy with a clinically recognizable phenotype.

C21.3

Wiedemann-Steiner Syndrome: Expanding the phenotypic spectrum associated with KMT2A (MLL) mutations

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In 2012 members of our group reported that de novo mutations in the histone methyl-transferase MLL (renamed KMT2A) underlie a distinct phenotype of hypertrichosis, short stature, intellectual disability and a distinctive facial appearance consistent with a diagnosis of Wiedemann-Steiner syndrome in five individuals. Other phenotypic features observed were feeding difficulties, behavioural difficulties, skeletal abnormalities and cardiac defects.

We have now identified 39 individuals with mutations in KMT2A. Our cohort contains 37 sporadic individuals and a set of monozygous twins. In total 19 individuals with KMT2A mutations have undergone detailed clinical phenotyping by members of our group and we have detailed phenotype information for 20 further patients with KMT2A mutations. Recruitment to our study, sequencing and detailed phenotyping is on going.

Our study has shown that hypertrichosis, which was initially identified, as a typical feature is variable in individuals with KMT2A mutations. We have expanded the list of phenotypic abnormalities associated with KMT2A mutations and report novel features including seizures, renal abnormalities, plantar fat pads, ptosis, and malrotation of the bowel. Review of the facial appearance of individuals of KMT2A mutations shows that the facial phenotype evolves over time, clinical photographs will be presented at different time points to illustrate this progression.

In addition to the expansion and clarification of the clinical features, growth profile and mutational spectrum associated with KMT2A mutations, we will review the overlap with other conditions and compare the phenotype of individuals identified through non-targeted screening to those individuals identified following clinician phenotyping to define the characteristic phenotype that should prompt consideration of KMT2A testing.

C21.4

Mutations in the endothelin receptor type A cause mandibulofacial dysostosis with alopecia via a maxillary to mandibular transformation

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The endothelin receptor type A (EDNRA) signaling pathway is essential for the establishment of mandibular identity during development of the first pharyngeal arch. We report four unrelated individuals with a novel syndrome, mandibulofacial dysostosis with alopecia (MFDA), who have de novo missense variants in EDNRA. The MFDA phenotype includes eyelid and ear dysplasia and hearing loss. Three of the four individuals have the same substitution in EDNRA, p.Tyr129Phe. Tyr129 is known to determine the selective affinity of EDNRA for endothelin 1 (EDN1), its major physiological ligand, and the p.Tyr129Phe variant increases the affinity of the receptor for EDN3, its non-preferred ligand, by two orders of magnitude. The fourth individual, previously described as having Johnson-McMillin syndrome, has a somatic mosaic substitution in EDNRA, p.Glu303Lys. The zygomatic arch of individuals with MFDA resembles that of mice in which EDNRA is ectopically activated in the maxillary prominence, resulting in a maxillary to mandibular transformation, suggesting that the p.Tyr129Phe variant causes an EDNRA gain of function in the developing upper jaw. Our findings highlight the importance of finely-tuned regulation of EDNRA signaling during human craniofacial development and suggest that modification of endothelin receptor-ligand specificity was a key step in the evolution of vertebrate jaws.

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C21.5
Mutations in transcription factor ZBTB20 cause tall stature, macrocephaly, cognitive deficits, diabetes, progressive muscle wasting and deafness

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Many Mendelian disorders with isolated or syndromic short stature have been recognized but the spectrum of conditions with increased growth appears much more restricted. Among the latter, the 3q13.31 microdeletion (del3q13.31) syndrome is a multisystem disorder characterized by increased postnatal growth, hypotonia, intellectual disability, disturbed behavior and unusual face. Primrose syndrome is an overgrowth condition characterized by macrocephaly, hypotonia, intellectual disability, autism and other behavioral concerns. Facial signs resemble del3q13.31 syndrome. Individuals with Primrose syndrome also develop diabetes in adulthood, progressive muscle wasting, hearing loss and ectopic calcifications. We used a WES-based strategy to identify the Primrose disease gene. We report that mutations in ZBTB20, residing within the 3q13.31 microdeletion syndrome critical region, underlie this disorder. Eight different missense mutations affecting residues located in the N-terminal region of the DNA binding domain of the transcription factor were identified. Mutations were predicted to affect DNA binding, and biochemical data provided evidence for reduced interaction with DNA and transactivation activity of disease-causing mutants. Mutations were documented affect protein function through a dominant negative action. Our findings establish a genetic link between this disorder and the clinically related del3q13.31 syndrome, and delineate the impact of ZBTB20 functional dysregulation and haploinsufficiency on development, growth and metabolism.

C21.6
Mutations impairing GSK3-mediated MAF phosphorylation cause cataract, deafness, intellectual disability, seizures, and a Down syndrome-like facies.

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Transcription factors operate in developmental processes to mediate inductive events and cell competence, and perturbation of their function or regulation can dramatically affect morphogenesis, organogenesis, and growth. We report that a narrow spectrum of mutations within the transactivation domain of the v-maf avian musculoaponeurotic fibrosarcoma oncogene homolog (MAF), a leucine zipper-containing transcription factor of the AP1 superfamily, profoundly affects development. Seven different de novo missense mutations involving conserved residues of the four GSK3 phosphorylation motifs were identified in eight unrelated individuals. The distinctive clinical phenotype, for which we propose the eponym Aymé-Gripp syndrome, is not limited to lens and eye defects as previously reported for MAF/Maf loss-of-function, but includes sensorineural deafness, intellectual disability, seizures, brachycephaly, distinctive flat facial appearance, skeletal anomalies, mammary gland hypoplasia, and reduced growth. Disease-causing mutations impair proper MAF phosphorylation, ubiquitination and proteosomal degradation, perturb gene expression in patient fibroblasts, and induce neurodevelopmental defects in an in vivo model. Our findings nosologically and clinically delineate a previously poorly understood recognizable multisystem disorder, provide evidence for MAF governing a wider range of developmental programs than previously appreciated, and uncover a novel example of protein dosage effect severely perturbing development.

C22.1
The secrets of GWAS are written in the reads

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Genome-wide association studies (GWAS) have identified a large number of disease associated loci, but in few cases have the functional variant and the gene it controls been identified. To systematically identify candidate regulatory variants we sequenced ENCODE cell lines and used public ChIP-seq data to find allele-specific transcription factor binding. We found 15,644 candidate regulatory SNPs of which more than 20% were rare, allele frequency <1%, and showed evidence of larger functional effect than common SNPs. This high frequency of rare functional variants adds heterogeneity to GWA studies of traits and expression and may explain divergent GWAS results between populations and why SNPs with the highest association signal rarely are functional. The majority of allele specific variants (95%) were specific to one of the six studied cell types. By examining GWAS loci we found >600 allele-specific candidate SNPs, 184 of which were highly relevant in our cell types. Results were confirmed by luciferase assays, EMSA and stimulation of primary cells. Functionally validated SNPs support identification of an intronic SNP in MERTK associated to risk for liver fibrosis, a SNP in SYNGR1 affecting risk for rheumatoid arthritis and primary biliary cirrhosis as well as a SNP in the last intron of COG6 affecting risk for psoriasis. We propose that by repeating ChIP-seq experiments of 20 selected transcription factors in three to ten people most common polymorphisms can be interrogated for allele-specific binding, in different cell types and tissues. Our strategy may help to alleviate the current bottle neck in functional annotation of the genome.

C22.2

Optimal ancestry-matched imputation of GWAS association summary statistics using large reference panel of sequenced individuals

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The ultimate goal of GWASs is to find disease-associated markers, preferably causal variants. GWASs use microarrays to interrogate tag SNPs, therefore many untyped variants need to be inferred through imputation, using the available genotyped SNPs. As demonstrated recently [Pasaniuc et al. (2014)], not only genotypes, but association summary statistics, generated by large meta-analytic studies, can be imputed.

We propose several improvements to state-of-the-art summary statistic imputation tools. We compute the optimal shrinking of the SNP-SNP correlation matrix, which flexibly adapts to the reference panel size, local LD structure, region size, etc. In addition, our method allows for summary statistics that are dervied from variable sample sizes. Finally, we search for the optimal linear combination of reference panel sub-populations in order to match their joint allele frequency distribution to that of the association studies. This is analogous to selecting the subset of the reference panel that best represents the individuals in the GWAS cohorts.

Our extensive simulation study, using UK10K whole genome sequencing data sets, showed that our optimized shrinkage constant significantly outperforms all previously proposed choices when the reference panel is small (<300). We also demonstrated the importance of ancestry weighted mixture using 1000 Genomes subpopulation panels combined with summary statistics simulated from our local CoLaus study (Firman et al., 2008). Finally, using cohort-level association summary data from the GIANT (Genetic Investigation of ANthropometric Traits) Consortium, we found that applying ancestry-matched imputation of individual cohort summary statistics before meta-analysis substantially outperforms the imputation of meta-analysed association summary statistics.

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C22.3

A novel method and software tool for genome-wide multi-phenotype analysis of rare variants

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Recently, genome-wide association studies (GWAS) have been expanded to analysis of low-frequency and rare variants (MAF≤5%, both denoted by RVs). Power for variant detection could also be increased by jointly analysing multiple correlated phenotypes. We have developed software for genome-wide Multi-phenotype Analysis of RVs (MARV), combining features from both RV burden tests and multi-phenotype analyses. Specifically, the proportion of rare variants at which an individual carries minor alleles within a gene region is modelled on linear combinations of phenotypes in a regression framework. MARV also implements model selection via the Bayesian information criterion (BIC). We have applied this new method on three correlated phenotypes: fasting insulin (FI), triglycerides (TG) and waist-to-hip ratio (WHR), using data from 4788 individuals from the Northern Finland Birth Cohort 1966. Individuals were genotyped on the Illumina370CNV array and imputed to the 1000 Genomes Project all ancestries reference panel (March 2012). FI/TG/WHR were adjusted for body mass index and three principal components to control for population structure. The following transformations were applied: natural logarithm for FI and inverse normal for the residuals of TG and WHR. We identified RV associations, at genome-wide significance ($p<1.7x10^{-6}$, Bonferroni correction for 30,000 genes) in *ZNF259*, which maps to a common variant GWAS locus for TG and coronary heart disease. Based on BIC, the model with TG and FI provided the best fit ($P_{model}=3.1x10^{-9}$), and stronger associations than in univariate analyses ($P_{TG}=6.7x10^{-8}$; $P_{FI}=0.13$). Using MARV, we demonstrate its ability to identify RV multi-phenotype associations with greater statistical significance than in univariate analyses.

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C22.4

The Exomiser suite for exome prioritization of human disease genes

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Introduction: Whole-exome sequencing has revolutionized rare disease research. However, many cases remain unsolved due to the fact that ~100-1000 loss of function, candidates remain after removing common and non-pathogenic variants. Exomiser offers a suite of algorithms that address this problem by additionally combining gene-based measures of candidacy. hiPHIVE (Human Interactome PHenotypic Interpretation of Variants in Exomes) assesses each candidate gene by comparing the patient phenotype to existing knowledge from disease and model organism databases. For genes with missing data, a guilt-by-association approach is applied, based on the phenotypic similarity of near-by genes in a protein-protein association network. PhenIX is designed for use in a clinical diagnostic setting where only genes associated with existing Mendelian diseases are phenotypically compared with the patient signs and symptoms. ExomeWalker ranks exome candidates based on their proximity in protein-protein networks to genes shown, or suspected, to be associated with the disease.

Results: In benchmarking experiments with known disease mutations added to unaffected exomes, both hiPHIVE and PhenIX were able to detect the causative variant as the top hit in 97% of samples. Further experiments, where knowledge of the known disease-gene association was masked, revealed hiPHIVE could detect novel associations as the top hit in 87% of samples. Exomiser is freely available via download for command-line use or through our web interfaces.

Conclusions: We are applying Exomiser to the NIH Undiagnosed Diseases Program and detect causative variants as the top hit in most previously solved cases and have diagnosed several new cases including a novel association.

C22.5

Allele specific expression reveals common and rare regulatory variation acting in human substantia nigra and putamen

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Allele-specific expression (ASE) occurs when the two alleles of a transcribed polymorphic locus are differentially expressed. The advantages of ASEs are: (1) they are a within individual comparison, so this avoids potential confounding factors; and (2) they can be interrogated in small sets, even single samples, from rare tissues. ASEs can therefore help clinicians gauge the pathogenicity of rare variants obtained from exome sequencing.

As part of the UKBEC project, we applied ASE analysis to paired RNA and exome sequencing data from 84 substantia nigra and putamen samples ob-

tained from 53 neuropathologically normal post-mortem human brains. We analysed mRNA-enriched (but not mRNA-exclusive) total RNA, to explore gene expression in both pre-mRNA and mRNA.
7.83% of the heterozygous variants we studied were identified as ASE signals at a False Discovery Rate < 5%. We found 66% concordance with the lymphoblastoid cell line data of Lappalainen *et al.* (2013), giving strong validation considering the difference in tissues in both datasets. Signals that showed a reversal in direction of ASE between individuals were seen to be enriched for known imprinted genes. Additionally, 91 ASE sites that are also known risk loci for a range of disorders and phenotypes were also identified, of which 64.84% were exonic and 30.76% were intronic SNPs. Amongst ASE sites identified, 15% are risk loci for adult neurological disorders thus providing insights into these disorders and indicating the power of this approach.

C22.6
Evidence for directional dominance on complex traits relating to size and cognition in a wide range of human populations

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Homozygosity has long been associated with rare, often devastating, Mendelian disorders and Darwin was one of the first to recognise that inbreeding reduces evolutionary fitness. However, the effect of the more distant parental relatedness common in modern human populations is less well understood. Genomic data now allow us to investigate the effects of homozygosity by measuring runs of homozygosity (ROH), however information is required on very large numbers of people to provide sufficient power. Here we use ROH in a study of 16 health-related quantitative traits in up to 354,224 individuals from 102 cohorts and find statistically highly significant associations between individual genome-wide summed ROH and four complex traits: height, forced expiratory lung volume in 1 second (FEV1), general cognitive ability (g) and educational attainment (nominal p<1 x 10-300, 2.1 x 10-6, 2.5 x 10-10, 1.8 x 10-10). In each case increased homozygosity was associated with decreased trait value, equivalent to the offspring of first cousins being 1.2 cm shorter and having 10 months less education. Similar effect sizes were found across four continental groups and in populations with different degrees of genome-wide homozygosity, providing convincing evidence for the first time that homozygosity, rather than genetic or environmental confounding effects, contributes to observed phenotypic variance. Directional dominance is predicted for traits under directional evolutionary selection, so this study provides evidence that increased stature and cognitive function have been positively selected in human evolution, whereas many important risk factors for late-onset complex diseases have not.

C23.1
TBK1 mutations cause amyotrophic lateral sclerosis and fronto-temporal dementia

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Amyotrophic lateral sclerosis (ALS) is a genetically heterogeneous neurodegenerative disorder hallmarked by adult-onset loss of motor neurons and fatal paralysis. Mutations in 29 genes have been linked to ALS pathogenesis and are frequently also associated with fronto-temporal dementia (FTD). However, mutations in these genes explain less than 1/3 of ALS cases.
To evaluate the contribution of low-frequency variants in protein-coding genes to familiar ALS, we performed exome sequencing of 252 index cases and 827 control individuals. We performed gene-based rare variant analysis and identified a single exome-wide significant enrichment of loss-of-function (LoF) mutations in the gene TANK-binding kinase 1 (TBK1) in the patient group. Seven different LoF mutations were found in nine patients, whereas LoF variants were absent in controls and in 3,101 additional in-house exomes.
No enrichment of LoF mutations was detected using a targeted mutation screen of 1,010 sporadic cases and 650 Swedish control individuals.
Next, we recruited relatives from index mutation carriers to perform linkage analysis. Incomplete penetrance was observed in all pedigrees. Linkage analysis in 3 extended families using a dominant model with reduced penetrance resulted in a summary LOD score of 4.6 thus exceeding the genome-wide significance threshold.
In vitro experiments including patient-derived cell lines confirmed loss of expression of TBK1 LoF mutant alleles, or loss of interaction of the TBK1 coiled coil domain (CCD) with the TBK1 adaptor protein optineurin, an established ALS target.
In conclusion, we provide parallel evidence from association testing and linkage analysis that LoF TBK1 variants cause a monogenic form of ALS with FTD and reduced penetrance.

C23.2
PMPCA Mutations cause Abnormal Mitochondrial Protein Processing in Patients with Non-Progressive Cerebellar Ataxia

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Non-progressive cerebellar ataxias (NPCAs) are a rare group of disorders, with only 7 genes/loci described, for autosomal recessive NPCAs. NPCAs manifest in infancy with abnormal gross motor development and hypotonia, followed by the appearance of ataxia. Dysarthria, intellectual disability and spasticity are often present.
We studied 17 patients from 4 families affected with NPCA, including the large Lebanese family previously described by Megarbane et al (1999) and localized to chromosome 9q34 (Delague et al, 2001; OMIM #213200). Homozygosity mapping and exome sequencing led to the identification of mutations in PMPCA in all patients: a homozygous p.Ala377Thr missense mutation in 16 patients, and compound heterozygous mutations, p.Ser96Leu and p.Gly515Arg, in one. We describe a founder effect for the p.Ala377Thr mutation, as patients from 3 a priori non-related Lebanese families share a common haplotype at the PMPCA locus.
PMPCA encodes α-MPP, the alpha subunit of mitochondrial processing peptidase (MPP), the primary enzyme responsible for the maturation of nuclear-encoded mitochondrial proteins. Analysis of cells from patients homozygous for the PMPCA p.Ala377Thr mutation and carriers, demonstrate that the mutation impacts both the level of α-MPP and the function of MPP. Indeed, this mutation impacts the maturation process of frataxin, the defective protein in Friedreich ataxia.
Our results definitely implicate PMPCA as the causal gene in this rare form of NPCA. This is the first time that the MPP enzyme, vital to life at the cellular level, has been associated with a clinical phenotype in humans. Disruption of mitochondrial protein precursor cleavage represents a new avenue for investigation of the pathogenesis of NPCAs.

C23.3

Spinocerebellar ataxia type 28, from molecular hypothesis to human therapy

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Spinocerebellar ataxia type 28 (SCA28) is a neurodegenerative disease caused by mutations of the AFG3L2 gene. The encoded protein assembles into multimeric complexes (the m-AAA proteases), which exert protein quality control in the inner mitochondrial membrane and participate to the regulation of mitochondrial morphology. The Afg3l2 haploinsufficient mouse recapitulates the symptoms of SCA28 patients, presenting a progressive decline in motor skills caused by dark degeneration of Purkinje cells (PC-DCD) of mitochondrial origin. In this work, we define the pathogenetic mechanism of SCA28 and provide the first evidence of a pre-clinical treatment of this disease. We demonstrated in cultured PCs that an inefficient buffering of stimulus-evoked Ca2+ peaks by Afg3l2-deficient mitochondria provokes an increase in cytoplasmic Ca2+ concentration, thus triggering PC-DCD. Proving this mechanism, we completely recover the ataxic phenotype of SCA28 mice by genetically reducing the metabotropic glutamate receptors mGluR1, and thus decreased Ca2+ influx in PCs. The same result has been successfully replicated by administration of an off-label therapy with ceftriaxone that favors the synaptic glutamate clearance. This treatment is effective when applied at both presymptomatic and after the ataxia onset in the preclinical model, thus representing a safe and immediately accessible therapy for presymptomatic carriers of AFG3L2 mutations and also SCA28 patients with overt symptoms.

C23.4

Homozygous truncating mutations in WDR73 cause a severe nephrocerebellar syndrome, part of the Galloway Mowat syndrome spectrum

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We investigated 30 Amish individuals (1-28yrs) with a severe nephrocerebellar syndrome characterised by progressive microcephaly, visual impairment, stagnant psychomotor development, abnormal extrapyramidal movements, and steroid resistant nephrosis. Post-mortem neuropathology revealed micrencephaly and atrophic cerebellar hemispheres with a unique pattern of histological findings. Assuming autosomal recessive inheritance of a founder mutation we used autozygosity mapping and next generation sequencing to identify the underlying molecular cause, a homozygous frameshift variant in WDR73 (c.888delT; p.Phe296Leufs*26). Interestingly, a second truncating frameshift variant (c.1264_1270delATAAAAG) (NM_001080435.2) was also identified in the closely linked WHAMM gene, which was found to be homozygous in all but one affected individual. A further novel homozygous frameshift variant (WDR73 c.766dupC; Arg256Profs*18) was identified in a Bulgarian child with similar clinical features. Our functional studies revealed that wild type WDR73 protein is expressed in human cerebral cortex, hippocampus, and cultured embryonic kidney cells and interacts with α- and β-tubulin, heat shock protein 90 (HSP-90), and CAD; the mTORC1-regulated multi-enzyme complex. We show that WDR73 protein is concentrated at mitotic spindles and midbody microtubules during mitosis, and recombinant WDR73 mutant (p.Phe296Leufs*26, p.Arg256Profs*18) proteins are unstable displaying increased interaction with α- and β-tubulin and HSP-90. Together, our data confirm that mutation of WDR73 is responsible for a complex nephrocerebellar syndrome best classified as part of Galloway Mowat syndrome spectrum of disorders. However given that the phenotypical outcome of WHAMM mutation is currently unknown, patients doubly homozygous for WHAMM mutation in association with WDR73 mutation may exhibit a composite phenotype.

C23.5

Mutations in PDE10A, resulting in a loss of PDE10A activity cause a hyperkinetic movement disorder in humans and in a mouse model.

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Clinical trials with phosphodiesterase 10A (PDE10A) inhibitors are currently underway in both Huntington's disease and Schizophrenia. PDE10A inactivates the critical intracellular signalling molecules Cyclic AMP and cyclic GMP; it is specifically expressed in the medium spiny neurons (MSNs) of the striatum.
In animal models PDE10A inhibitors ameliorate symptoms of schizophrenia, and the striatal pathology and clinical signs of HD, providing the rationale for these clinical trials in humans. However, very little is known about the role of PDE10A in man.
We observed two families with a very distinctive phenotype of a hyperkinetic movement disorder, variable mental retardation and abnormal skin pigmentation.
The pedigree structure of both families suggested a recessive disorder. Whole genome autozygosity mapping and exome sequencing revealed the presence of different homozygous mutations in PDE10A. Modeling of one of the mutations in cell lines showed that there was a reduction in protein levels and abnormal localization of PDE10A to the cytosol rather than the cell membrane where it is required for its activity.
We constructed a knock in mouse with one of the variants, this model animal displayed deficient motor control. The animal had reduced levels of PDE10A protein in the striatum, and furthermore there was a marked reduction in striatal PDE10A activity, and a loss of downstream signaling activity.
These data show that in humans congenital loss of PDE10 activity results in a movement disorder and variable cognitive impairment

C23.6

PLP1 mutations affecting *PLP1/DM20* alternative splicing causes Hypomyelination of Early Myelinating Structures

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Inherited leukodystrophies represent a diagnostic challenge and many patients remain without a diagnosis. In this study we investigated the genetic etiology of the recently described X-linked disorder 'Hypomyelination of Early Myelinating Structures' (HEMS) in 16 patients diagnosed by brain MRI criteria. Using exome sequencing, we identified in all patients unusual hemizygous mutations in the *PLP1* gene, located either in exon 3B (1 deletion, 1 missense and 2 silent), which is spliced out in isoform DM20, or in intron 3 (5 mutations). The frameshift deletion led to truncation of PLP1, but not DM20. *In silico* analysis of effects of the mutations on splicing and secondary RNA folding showed that four mutations located deep in intron 3 were predicted to destabilize a long-distance interaction structure in the secondary *PLP1* RNA fragment involved in regulating *PLP1/DM20* alternative splicing. The other four mutations were predicted to alter *PLP1/DM20* alternative splicing, either by creating exonic splicing silencers motifs, a splice donor site or by affecting the local RNA structure of the *PLP1* splice donor site. *In vitro* studies confirmed a decreased *PLP1/DM20* ratio in patients'

fibroblasts and transfected immortalized immature oligodendrocytic cells. Intriguingly, in patients with HEMS, brain structures that normally myelinate early, are hypomyelinated, in contrast to Pelizaeus-Merzbacher disease, also caused by *PLP1* alterations. This suggests that *PLP1/DM20* alternative splicing is important for early myelination, probably by an impact on the *PLP1/DM20* ratio. Our data extend the phenotypic spectrum of *PLP1*-related disorders and support the need to include intron 3 in diagnostic sequencing.

POSTERS

PS01.01

Clinical experience with a SNP-based noninvasise prenatal test for 22q11.2 deletion syndrome

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Objective: In contrast to whole chromosome aneuploidy, microdeletion syndromes are smaller and thus harder to detect, and they occur with equal frequency in women of different maternal ages. Here we report on the clinical experience with our single-nucleotide polymorphism (SNP)-based noninvasive prenatal test (NIPT) for the microdeletion responsible for the 22q11.2 deletion syndrome (DiGeorge).
Method: 21,948 maternal blood samples received between February and August 2014 were analyzed for the 22q11.2 deletion. Cell-free DNA and maternal-specific DNA were isolated from the samples. 672 SNPs in a 2.91 Mb DNA segment commonly deleted in the 22q11.2 deletion syndrome were PCR-amplified, sequenced, and analyzed using a proprietary algorithm to determine fetal and maternal copy number at the interrogated region. Follow-up information was sought for all high-risk cases.
Results: In the study cohort, 97 (0.5%) patients were found to be at high risk for a 22q11.2 microdeletion, including two cases in which the mother was suspected to have the deletion. Fetal diagnostic confirmation was available for 58 high-risk cases: 11 were true positives and 47 were false positives, resulting in a positive predictive value (PPV) of 19%. Invasive testing decisions were available for 81 high-risk cases: 60.5% had invasive testing and 39.5% declined. Ultrasound abnormalities were confirmed for 81.8% of true-positive and 10.6% of false-positive cases. Follow-up is ongoing.
Conclusions: Despite the small size of the 22q11.2 microdeletion, this SNP-based NIPT was highly effective and may be considered as a first-line approach for the general pregnancy population, not just for high-risk patients.

PM01.02

Complete 46,XY female of 14 cases and review of the literature

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Introduction : The individual of phenotypically female with 46,XY karyotype is caused by various abnormality such as gonadal formation and androgen synthesis and sensitivity during sexual development. XY female individuals are rarely found and have complex features. The degree of secondary sexual characteristics, level of hormone and presence or absence of Müllerian organ might be shown by various causes.
Materials and Methods : Cytogenetic analysis were performed in 9,472 female patients at Cheil General Hospital between September 1983 and December 2014. Clinical findings, basal hormone profiles, radiological readings were investigated. Additionally, DA-DAPI staining, CBG-banding, quantitative fluorescence-polymerase chain reaction analysis, fluorescence in situ hybridization were conducted to confirm the presence of Y chromosome and *SRY* gene.
Results : Among the 9,472 individuals, 14 cases found with complete 46,XY female (0.0015 %). Indications of 12 cases were primary amenorrhea (86 %) and two cases were agenesis of uterus and ovary cyst dysgeminoma respectively. Six cases had no uterus and high levels of testosterone. Four cases were performed gonadectomy. Four cases were familial. Only one case was diagnosed as androgen insensitivity syndrome.
Conclusions : Although further genetic testing and research is needed for an accurate diagnosis, the present report would be helpful for genetic counseling of XY female patient and their family, and understanding the one of the genetic etiology as the causal factor in amenorrhea.

PS01.03

aCGH in three fetus with severe intrauterine growth retardation (IUGR)

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Background:
The application of aCGH technology in prenatal diagnostics enables the detection of submicroscopic copy number changes that are associated with clinically significant outcomes.
Objective:

We report three cases of severe intrauterine growth retardation due to rare chromosomal aberrations diagnosed by aCGH. Prenatal and postnatal phenotypes are presented.
Methods and Results:
Fetal growth retardation as the leading finding was seen on ultrasound in the second/third trimester of pregnancy. In each case uterine artery Doppler was normal. With aCGH pathogenic submicroscopic copy number changes were identified, not detectable by cytogenetic chromosomal analysis: a) a terminal 11.4 megabase (Mb) deletion in 1q43q44, b) a 11 Mb duplication in 10p15.3p14 and a 1.9 Mb deletion in 17p13.3, c) a duplication in 14q11.2q12 (8.3 Mb) and deletion in 15q11.2q12 (3.2 Mb). While the copy number changes in cases a and b were de novo events, a pure deletion 1q43q44 in case a and a de novo translocation der(17)t(10;17)(p13;p13) in case b, the imbalances in case c resulted from an unbalanced inheritance of a reciprocal translocation t(14;15)(q12;q12). Beside growth retardation, the three young patients showed multiple dysmorphic features und further abnormalities after birth. For all, a distinct developmental delay/mental retardation is expected.
Conclusion:
aCGH should be adopted as a first-tier genetic test in fetus with idiopathic IUGR before routine G-banded karyotyping. The incremental information provided by aCGH allows prognosis prediction and hereby improves parental counselling.

PM01.04
Mutation analysis of androgen receptor gene:Multiple uses for a single test

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Abstract: Androgen receptor gene mutations are one of the leading causes of disorders of sex development (DSD) exhibited by sexual ambiguity or sex reversal. In this study, 2 families with patients whom diagnosed clinically as androgen insensitivity syndrome (AIS) were physically and genetically examined. This evaluation carried out by cytogenetic and molecular analysis including karyotype and sequencing of SRY and AR genes. In family1, two brothers and their mother were hemizygous and heterozygous respectively for c.2522G>A variant, while one of their healthy brother was completely normal hemizygous. Family 2 assessment demonstrated the c.639G>A (rs6152) mutation in two siblings who were reared as girls. The SRY gene was intact in all of the study's participants. Our findings in family 1 could be a further proof for the pathogenicity of the c.2522G>A variant. Given the importance of AR mutations in development of problems such as sex assignment in AIS patients, definitive diagnosis and phenotype-genotype correlation could be achieved by molecular genetic tests that in turn could have promising impacts in clinical management and also in prenatal diagnosis of prospect offspring.
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PS01.05
Genome wide association analysis of Anti Müllerian Hormone (AMH) in about 1,300 caucasian women highlights 2 novel suggestive loci for fertility.

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AMH is a dimeric glycoprotein, member of the TGF-β superfamily expressed in the growing follicles of the ovary. AMH concentration in serum essentially reflects the ovarian follicular pool. AMH levels vary broadly in women during reproductive life, until after 40 years old when the AMH level starts to decrease sharply, becoming undetectable after menopause. The discovery of genetic variants responsible for the high variability in AMH level could be a useful marker to predict fertility and age of menopause.
AMH levels were measured in serum of 850 healthy women collected by the Italian Network of Genetic Isolates (INGI) and of 461 fertile women collected by Obstetrics and Gynecology Unit of San Raffaele Hospital, Milano.
A meta-analysis for AMH adjusted for age of 1311 samples was performed on genotypes imputed to the low variants enriched 1000G panel. Two suggestive loci were identified: a locus on chromosome 6 already associated to menopause (MAF 0.017; p=2.081E-07) and a second novel locus on chromosome 11 (MAF 0.012; p= 1.34E-07). A subset of 941 women was genotyped by high-coverage exome chip. A SKAT meta-analysis was performed to highlight rare variants in the coding regions associated to AMH levels. A new locus on chromosome 16 reached a suggestive p-value of 2E-05.
Meta-analysis will be enlarged by additional samples in order to increase the statistical power of the analysis and confirm the suggestive loci.

PM01.06
BACs-on-Beads technology and next-generation sequencing for aneuploidy screening in trophectoderm cells of human blastocysts

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Introduction: Preimplantation genetic testing is a technique used to identify genetic defects in embryos created through in vitro fertilization (IVF) before pregnancy. Array comparative genomic hybridization (aCGH) was the first technology to be widely available for provide more information by analyzing all chromosomes at one time. However, the throughput and cost of aCGH may limit widespread application in clinical laboratories. The purpose of this study was to investigate the effectiveness of a new rapid aneuploidy screening test based on BACs-on-Beads (BoBs) technology and next-generation sequencing (NGS).
Materials and Methods: Trophectoderm (TE) samples of 10 blastocysts were subjected to analysis. The Karyolite™ BoBs kit was used to study aneuploidies involving any of the 24 chromosomes. Low-coverage whole genome sequencing was performed using the Ion Torrent PGM with 316 chip. The efficiency of these both approaches were estimated by comparing results obtained by aCGH.
Results: Whole genome amplification (WGA) products of TE cells were detected by both BoBs and NGS technology. One embryo (10.0%) was detected as euploid, while three embryos (30.0%) contained single chromosomal aneuploidy. Six of these (60.0%) were with multiple chromosomal abnormalities. The results from both technologies were compared with aCGH revealed that both methods were concordance 100% sensitivity and specificity.
Conclusions: Our study demonstrated both BACs-on-Beads technology and next-generation sequencing could be applied to accurately detect embryonic chromosomal abnormality with a flexible and cost-effective strategy and higher potential accuracy.

PS01.07
Maintenance of the methylation pattern on fresh and cultured Chorionic Villi (CV) in normal and Beckwith Wiedemann Syndrome (BWS)-suspected pregnancies

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BWS is an imprinting-related disorder that can be prenatally suspected following established clinical guidelines. Molecular confirmation is commonly performed on amniocytes and the possibility to use fresh (CVF) and cultured (CVC) CV has never been investigated.
To verify whether CVF and CVC are eligible sources of DNA, we tested by pyrosequencing in normal pregnancies the methylation percentage at: ICR1, ICR2, H19 promoter, PWS/AS-ICR, MGMT and RASSF1A genes. We highlighted stable methylation levels at the imprinting-driving regions ICR1 (CVF: 45.38% ± 1.77; CVC: 45.04% ± 1.81), ICR2 (CVF: 44.32% ± 1.84; CVC: 43.67% ± 2.10) and PWS/AS-ICR (CVF: 43.70% ± 5.60; CVC, 43.15% ± 3.41). Conversely, H19 promoter was severely hypomethylated at both CVF (11.33% ± 1.92) and CVC (19.30% ± 4.30), and showed a significantly increased methylation after culture. In two unrelated and biallelic genes, the methylation remained stable at MGMT promoter and changed at RASSF1A.
As second step, we investigated ICR1 and ICR2 methylation level on both CVF and CVC of two BWS-suspected fetuses (P1 and P2). P1 showed hypomethylation at ICR2 both in CVF and CVC (CVF: 17.63% ± 0.88; CVC: 16,13% ± 0.18); P2 showed normal methylation profiles.
Taken together these findings suggest that: i) ICR1 and ICR2, but not H19,

are reliable targets for BWS prenatal methylation test in CV also after culture; ii) similarly, PWS/AS-ICR is steadily hemimethylated in CV from healthy pregnancies, independently from culture. Thus, methylation analysis of these regions represents a very useful tool for prenatal diagnosis of imprinting related syndromes.

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PM01.08

CDKN1C mutations in familial and prenatally diagnosed Beckwith-Wiedemann syndrome cases.

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CDKN1C (cyclin dependent kinase inhibitor 1) gene mapping within the 11p15.5 cluster of imprinted genes, encodes an inhibitor of several cyclin dependent kinases (Cdk) acting in the cell cycle G1 - S transition. The monoallelic maternal expression of CDKN1C is under the control of the IC2 imprinting centre through the antisense non coding KCNQ1OT1 transcript. The protein comprises three functional domains including from the N- to the C-terminus a CDK binding, a PAPA and a PCNA domain.

CDKN1C mutations underlie the Beckwith Wiedemann syndrome (BWS) accounting for 10% of sporadic cases and up to 40% of familial cases.

Here we report on the clinical and molecular characterization of ten BWS families, negative for the known 11p15 (epi)genetic alterations and 2 prenatal cases diagnosed because of omphalocele. Twelve distinct mutations were detected, including 6 stop, 3 frameshift, two missense mutations and one complex mutation, seven of which yet unreported. Most important 11/12 mutations were maternally transmitted allowing to provide the family with genetic counseling and to assess the recurrence in two subsequent prenatal diagnoses. The clinical presentation of the investigated cases was heterogeneous although severe in all the carriers of inactivating mutations, including one case born prematurely at 28th week also displaying psychomotor delay and another with autistic traits, not associated with de novo or inherited CNVs as shown by array CGH.

Conversely the two patients with a missense mutations, predicted to be damaging by several bionformatic tools, exhibited a mild phenotype

PS01.09

Expanded carrier screening of 311,688 individuals: the case for going beyond CF

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We report our experience performing expanded carrier screening for 98 recessive conditions on an ethnically-diverse group of 311,688 individuals primarily from the USA, using targeted genotyping and next-generation sequencing. Individuals of European ancestry comprise 65% of the sample. All tested diseases were categorized as "profound", "severe", or "moderate" by a recently published methodology [Lazarin et al., PLoS One 2014]. "Severe" diseases (e.g., cystic fibrosis) are those that cause intellectual disability or shorten lifespan to adolescence or earlier; "profound" diseases (e.g., Canavan disease) do both.

We find that in every ethnic group, there is a higher absolute risk of fragile X syndrome (FXS) than of spinal muscular atrophy, recommended for universal screening by the American College of Medical Genetics and Genomics; in European groups, FXS is at least 50% as common as CF, and has higher risk than CF in all non-European groups.

We find the absolute risk for "profound" disorders not on an ethnicity-specific panel to be 60-80% that of CF (classified as "severe") in all European populations. Non-CF "severe" disorders are even more common, from 3.9x CF risk in Ashkenazi to 99.3x in East Asians.

Our data show that the absolute risk for diseases in expanded carrier screening panels is comparable to or significantly higher than the risk detected by existing carrier screening guidelines, especially in non-Caucasian populations.

		Absolute risk relative to CF risk			
Ethnicity	Absolute risk of CF (1-in-X births)	Spinal muscular atrophy	Fragile X syndrome	Profound diseases	Severe diseases
African	12341	75%	300%	89%	518%
Ashkenazi	1745	14%	95%	131%	389%
Mixed/Other Caucasian	1473	16%	44%	63%	389%
Northern European	1622	19%	51%	60%	374%
Southern European	1987	29%	53%	80%	286%
Unknown	2371	23%	86%	75%	499%
Hispanic	4348	39%	115%	72%	656%
East Asian	143037	935%	911%	3554%	9926%
Southeast Asian	90603	806%	724%	925%	4680%
Middle Eastern	9522	103%	768%	106%	2623%
South Asian	7079	56%	248%	29%	1693%

PM01.10

p.Asn386Lys, a new putative cystic fibrosis mutation on a complex allele discovered during assisted reproduction explorations, with an unknown phenotypical impact

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Cystic fibrosis (CF) is an autosomal recessive genetic disease caused by mutations in the CF transmembrane conductance regulator (CFTR) gene. Almost 2,000 mutations have been described, resulting in CF and CFTR-related disorders including male infertility by congenital absence of the vas deferens (CBAVD). Here, we report a Portuguese couple undergoing assisted reproduction because of CBAVD. The man was compound heterozygous for the CF-mutation c.1000C>T (R334W) and the CFTR-RD associated complex allele c.[1210-34TG[13]T[5];3705T>G] (TG13T5;S1235R). The genotype was consistent with the phenotype. The partner carried three heterozygous variants: c.1727G>C (G576A) and c.2002C>T (R668C), which are known as a complex allele, per se not associated with CF but sometimes in association with a third mutation, resulting in a CF or CFTR-RD allele, and c.1158C>A (N386K), which was never reported previously. Family study led to characterize a new complex allele combining the three variants. Evaluation of a possible impact of the c.1158C>A (N386K) mutation by bioinformatic tools did not suggest an effect on splicing but showed a possible effect on the protein level. This mutation, in the context of a complex allele, was thus considered to be potentially associated with CF, although functional studies would help document such an effect. This makes genetic counseling cautious, with a 25% hypothetical risk for the couple of having a child with CF. This case report illustrates the interest of thorough CFTR gene studies in CBAVD couples requesting assisted reproduction in order to provide accurate genetic counselling.

PS01.11

An unusual presentation of a human chimera - case study and investigation of the underlying mechanism by SNP array analysis.

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Human XX/XY chimerism, resulting from the fusion of two different zygotes, is a rare finding and is usually identified in newborns with ambiguous genitalia. In the present study we describe an apparently normal male age 34 years who presented with infertility. Sperm analysis revealed a low sperm count with decreased mobility, a diagnosis of severe oligoasthenoteratozoospermia was made. The proband is otherwise healthy and has received no blood or bone marrow transfusions. Karyotype analysis of peripheral blood and subsequent FISH analysis of buccal cells showed a mixture of 46,XY and 46,XX cells. Several mechanisms have been proposed for the formation of chimerism including 1) tetragametic chimera resulting from postzygotic fusion of two separate zygotes, 2) dispermic fertilization of either an oocyte and its second polar body, and 3) dispermic fertilization of two female gametes arising from parthenogenetic division of a single oocyte. Comparative QF-PCR studies of the proband and both parents provided evidence of a genetic contribution in the proband from two different sperm but no evidence of a genetic contribution from a second ovum, consistent with mechanism 2 or 3. A comparative genotyping SNP array analysis was undertaken to try to further elucidate the underlying mechanism in this case. This genetic diagnosis posed a challenging genetic counselling issue but has not fundamentally altered the proposed ICSI treatment for this individual's infertility.

PM01.12

Reliability of chromosomal microarray-based analysis in CVS for the detection of cryptic chromosomal abnormalities and fetoplacental discrepancies

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The study of chorionic villi samplings (CVS) by conventional cytogenetics reveals chromosomal abnormalities in 12% of cases, and in 1-2% of pregnancies mosaicism is observed. In order to increase the diagnostic yield, new molecular techniques that offer higher resolution have been developed, such as chromosomal microarray-based analysis (CMA). Similar to cytogenetically visible chromosomal abnormalities, cryptic chromosomal abnormalities (CCA) may also be presented as confined placental mosaics, contributing to misinterpretations. The purpose of the present work was to determinate the frequency of CCA and confined placental mosaicism of CCA in CVS, and to evaluate the reliability of the strategy used.
We performed CMA in CVS of 50 pregnancies with normal karyotype or a balanced familial rearrangement, in both trophoblast and mesenchyma. Twenty two per cent of them were referred for ultrasound abnormalities and 78% for abnormal first trimester screening.
In 94% of the CVS, CMA results could be obtained from both tissues. The overall frequency of reportable non-mosaic CCA was 8,5%, and in 2% of samples a CCA was only found in trophoblast. All the CCA were diagnosed in the abnormal first trimester screening group.
Although the cohort presented is relatively small, it seems that CCA and placental mosaicisms of CCA present a frequency similar to that of cytogenetically visible chromosomal abnormalities during the first trimester of pregnancy. However, CCA do not seem to be generally associated with ultrasound abnormalities in the first trimester. The strategy used is reliable for the detection of placental mosaicisms of CCA.

PS01.13

The cleavage-stage bovine embryo is a valuable model to study chromosome instability in early mammalian embryogenesis

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In vitro fertilization (IVF) is a widely used infertility treatment procedure, but the IVF outcome may be influenced by the high rate of chromosome instability (CIN) found in human preimplantation embryos. Although CIN has been observed in recent studies, its precise etiology remains elusive. However, experimenting on human embryos is associated with ethical issues. In this study, we investigate the incidence of CIN in in vitro cleavage-stage bovine embryos, and provide substantial proof that bovine early embryogenesis is a valuable model for the study of underlying mechanisms leading to CIN in an in vivo research setting.
151 blastomeres from 25 cleavage-stage embryos were obtained on day 2 and day 3 post insemination (pi) and whole-genome amplified (WGA). Subsequently, the samples were hybridized on the Illumina Bovine HD BeadChip SNP arrays. We consequently applied a modified version of the siCHILD algorithm (siCHILD-bovine) and haplarithmisis for the data analysis.
From 25 embryos analyzed, 7 were uniformly diploid (28%), while 18 embryos had blastomeres with chromosomal anomalies (72%), of which 83% were mosaic. Out of 124 blastomeres available for the analysis, 31% were diploid, 47% had whole-chromosome abnormalities and 27% carried segmental aberrations. Moreover, segmental reciprocal gains and losses in sister blastomeres were identified in 3 embryos.
This study demonstrates that the nature of CIN in bovine cleavage-stage and human cleavage-stage embryos are comparable. Therefore, cattle can be employed as a model organism for CIN studies. Data acquired from such studies could be used to raise the success rate of human IVF outcome.
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PM01.14

EasyChip 8x15K: a new and useful tool for anomalies detection in low risk pregnancies

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Microarray chromosome analysis is becoming a more and more routinely test and its use in prenatal diagnosis has been discussed, raising controversial opinions.
In order to limit incidental findings (IF) and variants of unknown significance (VOUS) we designed EasyChip, a low resolution oligo 8x15K array with a resolution of 3-4Mb on genomic backbone, 500Kb on subtelomeric portions and 250Kb on 43 syndromic regions.
Syndromic regions were selected considering morbidity, penetrance (>75%) and etiological mechanisms.
We evaluate EasyChip on 48 samples with known anomalies, and all the imbalances were detected: 22 syndromic regions (86.7Kb-33Mb); 29 subtelomeric regions (233.8Kb-40Mb); 7 backbone regions (1.9Mb-5.5Mb).
A prospective study was carried out on 32 cases of prenatal samples from low risk pregnancies, tested with both EasyChip and a higher resolution platform (4x180K/8x60K).
The only positive result detected by both the platforms was consistent with a female foetus presented with mosaicism 45,X/46X,i(X)(q10).
EasyChip did not detect 7 VOUS on genomic backbone, ranged 222.1-579 .6Kb, evidenced by high resolution platform.
EasyChip is a useful tool in prenatal diagnosis for screening purposes, associated with karyotype. It can support the standard cytogenetic analysis for detection of submicroscopic imbalances, which could be lost especially when working on not optimal quality samples. Moreover, it can detect cryptic imbalanced subtelomeric rearrangements and microdeletions/duplications within 43 specific regions associated with high morbidity syndromes.
Such a design has the advantage, over higher resolution platforms, to limit the detection of VOUS or IF, which complicate genetic counselling increasing parental anxiety, without providing certitude in pregnancy outcome.

PS01.15

Tissue-specific mosaicism for CNV in human miscarriages

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Introduction: Extensive load of somatic copy number variants (CNVs) has recently been shown in human placenta, suggesting that it may be critical for normal gestation. However, it is unclear weather the entire placenta or just certain placental cell types promote this phenomenon. Also it may occur that prevalence of CNVs in one cell type may be essential for normal development, while in another - responsible for embryolethality. We aimed to investigate CNVs in the only two placental tissues available from blighted ova - cytotropholast (CT) and extraembryonic mesoderm (EM).
Materials and Methods: Placental tissues of 10 euploid miscarriages were investigated using Agilent 180K microarrays.
Results: Altogether 198 CNVs were detected: 91 in EM and 107 in CT, 24 (26%) and 50 (47%) of them were unique respectively (p=0.0032). Microdeletions and microduplications were equally represented in EM (11 vs. 13), while microdeletions prevailed in CT (41 vs. 9). Unique microdeletions significantly more frequently were detected in CT (p=0.0014). The candidate developmentally important genes involved in tissue-specific CNV in EM are PTPN18 (cell growth, differentiation), CFC1 (embryonic development), OCLN (vascular integrity), WIST1 (cell lineage determination and differentiation), TBX10 (embryonic cell fate and organogenesis), in CT - CTNNA3 (inhibitor of trophoblast invasion), PTPRR (cell growth, differentiation), TSPAN8 (cell development, growth, motility), miR-296 (angiogenesis), PCGF1 (embryogenesis).
Conclusions: The presented profiling of CNVs may be the hallmark of abnormal pregnancy, specifically blighted ova, as according to Kasak et al. (2015) normal gestation whole-placental material is, on the contrary, characterized by the prevalence of duplications. This study was supported by Russian Foundation for Basic Research, 14-04-32047.

PM01.16

Cytogenetics evaluation among infertile couples in a Southern Iranian population; a targeted survey

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Introduction: The role of cytogenetics service in diagnosis, risk management, and outcome of infertility is becoming more and more evident. An abnormal finding can have significant consequences to assisted reproductive techniques and fertility treatment, and provide a firm diagnosis to couples

with longstanding infertility. In the present study we investigated the status of cytogenetic evaluations performed on infertile couples from a southern population of Iran.
Methodology: Couples with a history of infertility were interviewed regarding their medical history, any referrals into cytogenetics clinic, and genetic counseling using a standard questionnaire.
Results: A total of 438 couples were included. The mean age was 24.8 ± 5.2 years. Consanguinity was found among 23.4% of the couples. The majority of the participants (98.3%) never performed any kind of cytogenetic testing and 97.2% of them were never referred for genetic counseling. Finally, 10% of the participants experienced at least one abortion and mental retardation in their familial history.
Conclusions: While chromosomal abnormalities are responsible for a significant portion of infertilities, miscarriages, and IVF/ICSI failure, just a negligible number of the infertile couples in our study are offered cyotogentics service. Our findings highlight a shortage accessibility of such services as well as a lack of education amongst the involved clinicians about the cytogenetic causes of infertility in our target population.

PS01.17
Identification and characterization of novel fetal specific differentially methylated regions on chromosomes 13, 18, 21 and X

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DNA methylation is a conserved epigenetic mark that involves the addition of a methyl group on carbon 5 of cytosines present in CpG dinucleotides. Furthermore, tissue specific methylation patterns are investigated as biomarkers for cancer and cell-free fetal DNA using various methodologies. Our group has previously confirmed the presence of methylation variability on differentially methylated regions (DMRs) on chorionic villus sampling (CVS) and non-pregnant peripheral blood

samples (WBF). Despite the DNA methylation variability, the validated set of DMRs was clearly distinguished between CVS and WBF samples, enabling for robust tissue specific methylation identification. In this study we aimed to expand the prenatal panel of DMRs, utilizing custom 1 million ultra-high resolution aCGH chip designed for chromosomes 13, 18, 21 and X on normal/abnormal CVS and WBF. A subset of the identified DMRs was selected according to established criteria and confirmed by utilizing methylated DNA immunoprecipitation (MeDIP) and real-time quantitative PCR. In total we confirmed the differential methylation status in 99 regions on chromosomes 13, 18 and 21 and two on chromosome X; the majority of the DMRs were found to be located on genes and associated with diseases. Interestingly, four regions on chromosome 21 have been found to be correlated with genes that may play a role in the pathophysiology of Down syndrome. In conclusion, our work provides an expansion in the biomarker panel available for NIPT for Down syndrome and can eventually provide the starting point towards the development of assays towards the detection of all common chromosomal aneuploidies.

PM01.18
Study of genetics of human disorders of sexual development in Ukraine in the frame of SCOPES 2013-2016: Joint Research Projects

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The project "Genetics of Human Disorders of Sexual Development" is funded by Swiss National Science Foundation and fulfilled by the University of Geneva Medical School (Switzerland), the Institute of Human Genetics (Poland), the Center of medical genetics and primary health care (Armenia) and the Institute of Molecular Biology and Genetics (Ukraine).
The goal is to identify mutations underlying unresolved DSD phenotypes - in novel DSD genes, or regulatory regions that lead to atypical gene expression. Identification of new genes involved in human sex determination and differentiation is carried out through exome sequencing and CGH microarray in parallel.
Ukrainian partner is participating in all stages preceding the exome sequencing: clinical data collecting (caryotype, family history, physical examination, ultrasound, hormonal status, surgery, histology), caryotyping, DNA samplings (proband, parents, siblings), SRY gene deletion detecting and SRY, SOX9, WT1, SF1, LHX9, RSPO1, FOXL2, WNT4, DMRT1, DMRT2 genes Sanger sequencing. During the first year a few main medical centres of Ukraine joined the project: Institute of endocrinology and metabolism of Komisarenko and Regional centres of medical genetics of Zaporizhzhya, Kherson, Chernigiv, Lutsk, Poltava, Zhytomyr, Khmelnitskyi.
For the first year Ukrainian part collected 38 DNA samples from 16 DSD cases - 15 of 46,XY DSD females and 1 of 46,XX DSD male.
We expect the research will provide the opportunity to develop new genetic tests for DSD diagnosis and to improve understanding of the molecular mechanisms of ovarian and testicular differentiation.
The results on the first cohort will be discussed in details.
SCOPES 2013-2016: Joint Research Projects

PS01.19
Pathogenic compound heterozygous mutations in the ERCC2 gene in a foetus with severe congenital ichthyosis and dysmorphic features: a case report

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Background Mutations in the ERCC2/XPD gene cause rare autosomal recessive NER (Nucleotide Excision Repair)- related diseases including trichothiodystrophy (TTD), cerebrooculofacioskeletal syndrome (COFS), Cockayne syndrome (CS) and xeroderma pigmentosum (XP), or a combination of XP/TTD, XP/CS or COFS/TTD. These diseases share a number of clinical features and encompass a wide spectrum of severity, in particular TTD, characterised by sulphur-deficient brittle hair, short stature, intellectual disability, microcephaly, facial dysmorphism and ichthyosis. To date, there have been few reports describing foetal cases with mutations in ERCC2. Case report We describe a male foetus, the second child of healthy unrelated parents, who died in utero at 28 weeks of pregnancy. The autopsy revealed severe harmonious intrauterine growth retardation (<3rd centile), delayed bone maturation, congenital ichthyosis, and facial dysmorphism including low insertion of the columella, beaked nose, large and low set ears, micrognathism and retrognathism and upslanted palpebral fissures. The hands showed retracted broad and tapering fingers with hypoplastic nails. Caryotype and array-CGH were normal. Exome sequencing revealed compound heterozygous nonsense and pathogenic missense mutations (p.Gln698* and p.Arg722Trp respectively) in ERCC2, that were consistent with the clinical features. One mutation was transmitted from each of the patient's parents, respectively. Functional studies are on going to test the patient's NER capacities, using standardized methods. Conclusion This case confirms the power of exome sequencing in the rapid identification of rare clinically non-recognisable diseases, in particular in the absence of clinical clues suggesting a diagnosis, as in severe congenital ichthyosis, a condition that includes several distinct subtypes with significant genetic heterogeneity.

PM01.20
Do people from the Jewish community prefer ancestry-based or pan-ethnic expanded carrier screening?

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Background: Ancestry-based carrier screening in the Ashkenazi Jewish po-

pulation entails screening for specific autosomal recessive founder mutations, which are rare among the general population. As it is now technically feasible to screen for many more diseases, the question arises whether this population prefers an ancestry-based offer or a pan-ethnic expanded carrier screening panel that goes beyond the diseases relatively frequent in their own population, and is offered regardless of ancestry.
Methods: An online questionnaire was completed by 145 individuals from the Dutch Jewish community (≥18 years) between April and July 2014.
Results: 64.8% were aware of the existence of ancestry-based carrier screening. Respondents were generally positive about screening and thought that several categories of diseases should be included. About half (54%) preferred pan-ethnic expanded carrier screening whereas 43% preferred ancestry-based screening. Reasons for preferring pan-ethnic screening included "everyone has a right to be tested", "fear of stigmatization when offering ancestry-based panels", and "difficulties with identifying risk due to mixed backgrounds". "Preventing high healthcare costs" was the main perceived barrier to pan-ethnic carrier screening among those in favour of ancestry-based screening.
Conclusion: These findings show that people from the Dutch Jewish community have a positive attitude regarding carrier screening in their community for a wide range of diseases. Costs were the main perceived barrier for pan-ethnic carrier screening panels. As costs of these panels are most likely to drop in the near future, it may be expected that these will receive more support in the future.
The study was funded by Netherlands Organization for Health Research and Development (grant no 209040001).

PS01.21

Detection of a novel mutation in FANCD2 gene in a fetus with unilateral kidney agenesis and anomalies of upper limbs

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Congenital anomalies affect 1% to 2% of newborns, and approximately 4-5% of those children have abnormalities of urinary tract and kidneys and 10% - have upper-extremity abnormalities. Combining of homozygosity mapping using SNP arrays and sequencing of target regions has been successfully applied for identification of mutations causing many autosomal recessive diseases in patients diagnosed postnatally.
We used SNP array karyotyping and next generation sequencing to clarify the etiology of a polymalformative syndrome detected prenatally. On US scan at 18th g. w. the fetus of a second gravida was found to have: mild growth retardation, right kidney agenesis and absent radii and thumbs of both arms.
SNP karyotyping with Illumina Human CoreExome-12 revealed a homozygous chromosome region encompassing 6.1 Mb in 3p: arr[hg19] 3p26.1p25.3p25.2(6206901-12352468)x2hmz. The gene FANCD2 mapped in the region and was found to be a good candidate for the fetal malformative syndrome. We used TruSight Cancer gene panel (Illumina) and the MySeq sequencing system. The fetus was found to be homozygous for a novel hypomorphic mutation in FANCD2 gene: p.Leu699del and both parents were found to be carriers of the mutation.
The identification of an anomaly compatible with life during pregnancy is a challenge for both prospective parents and the doctor. The anomalies require an accurate diagnosis and communication of relevant information to the family. Certain anomalies occur in isolation, whereas others are associated with systemic conditions. Prognosis of the developing fetus and reproductive decisions of the family largely depend on the causes behind the disease.

PM01.22

Perinatal outcome of fetal echogenic bowel

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Introduction: The aim of this study is to describe the association between fetal echogenic bowel (FEB) during the second trimester and adverse perinatal outcome and to compare additional findings that contribute to the pregnancy outcome
Material and methods: We conducted a retrospective case control study. Perinatal outcome of 262 cases of FEB, reported between 2007-2014, were compared to 2827 cases of pregnancies without abnormal sonographic findings.
Results: Of the 262 cases 36 cases (13.7%) were associated with maternal vaginal bleeding and 15 cases (8.6%) of 174 who underwent serology testing had evidence of CMV seroconversion during the pregnancy as oppose to 0.1% in both parameters in the control group. 73 cases (27.8%) had evidence of structural malformation. 122 cases underwent amniocentesis and karyotyping, three of which had chromosomal abnormalities (2.45%). The incidence of IUGR and fetal demise was 9.23% and 6.42% as oppose to 2.9% and 0.5% in the control group, respectively. Composite endpoint calculation demonstrated absolute risk increase for fetal demise, IUGR, SGA, low apgar score of 10% in the isolated FEB group as compared to isolated polyhydramnios group. Interestingly, elevated level of alpha-fetoprotein in the FEB group were significantly ($p<0.001$) associated with preterm delivery and fetal demise.
Conclusions: The presence of FEB is independently associated with an increased risk for IUGR and fetal demise. Elevated aFP in addition to FEB contributes to adverse perinatal outcome. This information should be considered when counseling patients after FEB is diagnosed.
Grant references: None.

PS01.23

Gene discovery in lethal fetal malformation phenotypes - the value of human knockouts

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Prenatal ultrasonography identifies an increasing number of undescribed malformation phenotypes. Little attention has been paid to using whole exome and genome sequencing strategies for gene identification in fetal disorders that are lethal in utero, because they are extremely rare, may appear to be sporadic, and Mendelian inheritance can be easily missed. Some lethal phenotypes, however, indicate an error of early development implying a major malfunction of a gene with a crucial role in cellular and developmental processes.
Hypothesizing that truncating autosomal recessive variants are an important cause of early human lethality, we select families with phenotype recurrence in sibs who died during pregnancy or after birth because of their malformations. We correlate the malformation pattern, confirmed by autopsy, to developmental pathways in embryogenesis. Genes with homozygous or compound heterozygous variants will be considered candidates. Those harboring truncating variants will be prioritized, since loss of function variants are more likely to be causal compared to other variant classes.
We present two novel prenatal malformation patterns indicative of the disruption of pathways involved in ciliary and midline defect phenotypes. The role in cell division of candidate genes identified suggests a link to these developmental pathways. Causality is supported by cross-species phenotyping.
Identifying mutations implicated in early fetal development will improve recurrence risk counseling and allow prenatal diagnosis for future pregnancies in affected families. Lethal fetal phenotypes may represent an important model to study the genetic basis of natural human knockouts and the roles of genes for which little to nothing is known.

PM01.24

Microarray analysis of fetal cells isolated from maternal blood

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Introduction: Limitations of contemporary techniques of prenatal diagnosis necessitate the need to develop new non-invasive prenatal test. The presence of microchimerism state during each pregnancy opened up new possibilities for fetal genome analysis. Despite of many studies there is still no consensus protocol for identification of fetal cells in the maternal blood. We aimed to compare the expression of maternal and fetus genomes to determine potential fetal microchimeric cells markers.
Materials and Methods: The experimental material consisted of 5 ml of peripheral blood from women in the second trimester of pregnancy, who under-

went amniocentesis procedure. Analysis was based on CD34 positive cells. Cells were isolated from blood samples and were subsequently cultured to obtain the targeted line of hematopoietic colonies. After the incubation period, single erythroid colonies were collected and analyzed by means of microarray technology using GeneChip Human Gene 1.0 ST Array.
Results: The analysis showed statistically significant differences in the expression of 958 genes between maternal and fetal cells. Most of the genes (n = 591) showed higher expression in the fetus relative to the expression of the mother's genome. A total of 367 genes showed decreased expression in the fetus. Initial protocols were developed for culture and identification of fetal cells in the maternal blood.
Conclusions: The use of cell culture enables multiplication of the cells and reduces the volume of maternal blood sample necessary for the analysis. Transcriptome analysis of cells shows expression of specific genes, which may be used as markers for the identification of fetal cells.

PS01.25
First Report of Prenatal Diagnosis for Severe Genodermatoses in Egypt

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Introduction: Genodermatoses are mostly severe inherited disorders. A great success in identifying responsible genes & characterizing mutations within such genes paved the road for DNA-based prenatal diagnosis. Examples of severe genodermatoses candidate for prenatal diagnosis include autosomal recessive congenital ichthyosis (ARCI), Xerodema pigmentosa (XPA), Sjögren-Larsson syndrome (SLS) and papillon lefeuvre syndrome (PLS) where clinical severity affects span &/or quality of life hence urging prenatal diagnosis.
Materials and methods: The study included five amniotic samples (AF) from carrier mothers descending from five pedigrees with history of affected sibs with severe genodermatoses including; two mothers of previous ARCI cases, one XPA, one SLS and one PLS. DNA was extracted from AF samples by QIA gene extraction kit followed by mutational screening for XPA, TGM1, ALDH3A2 and CTSC genes.
Results: prenatal diagnosis was successfully performed in all cases. For the family with history of XPA, the fetus was found to be heterozygous carrier for E111X mutation; For ALDH3A2 gene the fetus was affected for E331X nonsense mutation; for TGM1 gene the two fetuses were heterozygous carriers for R264W, R143H missense mutations. The fifth AF sample PLS showed homozygous wild type genotype.
Conclusion: The high incidence of consanguinity & consequently AR rare disorders combined with the lack of curative therapy, points to the importance of implementing preventive programs. Prenatal diagnosis and genetic counseling represent an important step in prevention & alleviating the burden of severe genodermatoses on the family & community.

PM01.26
Incremetal yield of Array Comparative Genomic Hybridization above karyotyping in Fetal Increased Nuchal Translucency - A systematic review and meta-analysis

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Objective: To perform a systematic review of the literature and meta-analysis and estimate the incremental yield of genomic microarray over karyotyping in fetuses with increased nuchal translucency (NT) diagnosed by first trimester prenatal ultrasound.
Method: All articles identified in PubMed and Ovid, from January 2009 to September 2014 describing copy number variants (CNVs) in fetuses with increased NT were included. Search terms were: fetal or prenatal, nuchal translucency or cystic hygroma or ultrasound anomaly, array comparative genomic hybridization or copy number variants, with related search terms. Case reports and studies using conventional comparative genomic hybridization were excluded.
Results: Seventeen publications met the inclusion criteria for the analysis. A 5% (95% CI 2.0-8.0) incremental yield by microarray was obtained pooling the results. Stratified analysis demonstrated a 4% (95% CI 2.0-7.0) incremental yield for isolated NT and 7.0% (95% CI 2.0-12) when other malformations are present.
Conclusion: The review found that the use of genomic microarray provides a 5% incremental yield in fetuses with increased NT and normal karyotype.

PS01.27
Role of hereditary thrombophilia and antiphospholipid syndrome in pregnancy complications and recurrent miscarriages in IVF programs

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Introduction: Screening for hereditary thrombophilia and APS in women undergoing IVF proved to reduce miscarriage and pregnancy complications risk, to minimize thrombotic complications.
Materials and Methods: 420 women with pregnancy loss or preterm birth after IVF were examined for hereditary thrombophilia and APS. Women diagnosed with these conditions were administered antiaggregant therapy (aspirin, low-molecular-weight heparin), high doses of folic acid (5 mg daily), vitamin B12 (in cases of MTHFR gene polymorphism and elevated homocysteine concentration).
Results: 232 women (55.2%) were diagnosed with thrombophilia - 145 of them had hereditary thrombophilia (34.5%), 41 of them presented elevated homocysteine levels (9.8%); APS was found in 87 women (20.7%). The structure of hereditary thrombophilia carriers (145 cases): heterozygous mutation 1691G>A Leiden in F5 gene - 11 women (7.6%), heterozygous polymorphism G20210A in F2-prothrombine gene - 6 (4.1%), compound heterozygous carriers of 1691G>A Leiden + G20210A - 3 (2.1%), homozygous carriers of 677T/T in MTHFR gene - 38 (26.2%), compound heterozygous carriers of 677T + 1298C in MTHFR gene - 87 (60%). IVF with appropriate thrombotic complications prophylaxis was performed for 145 women with hereditary thrombophilia, 67 cases resulted in pregnancy (46.2%). Pregnancies ended with term birth - 46 (68.7%), preterm delivery (32-36 weeks) - 12 (17.9%), reproductive failures - 9 (13.4%), which included 5 miscarriages in 1st trimester (7.5%), 3 in 2nd trimester (4.4%), 1 ectopic pregnancy (1.5%).
Conclusion: Screening for hereditary thrombophilia and APS reduces risk of miscarriages and pregnancy complications in IVF procedures; and should be widely recommended, especially for women with thrombotic complications or reproductive failures in anamnesis.

PM01.28
Association of a HRG polymorphism with ovarian reserve and response to ovarian stimulation in women undergoing assisted reproductive treatment

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Histidine-rich glycoprotein (HRG) is a plasma protein involved in many biological processes, including fibrinolysis, coagulation, apoptosis and angiogenesis. These processes are important in oocyte development
and pregnancy. In a recent Swedish study, a homozygous variant in *HRG*, c.633C>T was associated with poor ovarian response during the assessment of women undergoing assessment for *in vitro* fertilization (IVF).[1]
The aim of our study was to investigate whether *HRG* c.633C>T is associated with the ovarian reserve markers follicle stimulating hormone (FSH), antral follicle count (AFC), and anti-Mullerian hormone (AMH) and ovarian response (number of eggs retrieved, and gonadotropin dose required) to controlled ovarian hyper-stimulation.
We genotyped *HRG* c.633C>T in 517 women, attending a tertiary referral centre for reproductive medicine, undergoing their first cycle of controlled ovarian hyper-stimulation for IVF/ICSI. We found no evidence of any significant difference (p value <0.05) in FSH, AFC, AMH, the number of eggs retrieved, or the gonadotropin dose used between individuals with different *HRG* genotypes.
These results indicate that this variant does not provide clinically relevant data on which to base the individualization of the treatment of women undergoing IVF/ICSI.
Ref. 1.

PS01.29

Genetic associations for hypospadias: review of current knowledge and replication

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Background
Hypospadias is a common congenital malformation of the male external genitalia with a multifactorial etiology. We identified all genetic associations reported for hypospadias and tried to replicate the most promising ones.
Methods
After a thorough literature search and SNP selection, we used Taqman assays to genotype seven SNPs in 816 Caucasian nonsyndromic hypospadias patients and 668 population-based controls derived from the AGORA data- and biobank in the Netherlands.
Results
When excluding studies using microarrays performed after our SNP selection, 36 polymorphisms in *DGKK* and 67 polymorphisms in 16 other genes were identified. We already examined *DGKK* successfully in our cohort and this gene was also associated with hypospadias in several other Caucasian populations, but much less strong in a Chinese. Many of the polymorphisms in other genes were found not to be associated with hypospadias, had a MAF<2% in Caucasians, or were repeats or deletions, while four SNPs had already been studied in our cohort. Therefore, we selected seven SNPs in the genes *HSD17B3*, *ESR1*, *ESR2*, *ATF3* and *MAMLD1* for this study. None of the SNPs was associated with hypospadias, with the possible exception of rs944050 in *ESR2* (OR=1.5, p=0.045). This association was in the same direction as in a Swedish study and in the opposite direction compared to a Japanese study.
Conclusions
Summarizing all currently reported genetic association studies on hypospadias shows that risk polymorphisms differ between populations. This stresses the importance of studying generalizability of genetic association results and points towards the need for gene-environment interaction analyses.

PM01.30

A family with deafness-infertility syndrome presenting in two generations

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Only few families with deafness and infertility syndrome, a contiguous gene deletion syndrome at 15q15.3 locus, have been described so far.
It is rare and inherited as an autosomal recessive trait (homozygous for the deletion females present with deafness whereas males with infertility and deafness).
We present a family with two generations affected by sensorineural hearing loss (mother and two sons). Both sons were diagnosed with infertility, one of them had a child through assisted reproductive technology.
Using the array CGH method the deleted region in the proband has been described to encompass 55 kb. It includes the gene *CATSPER2* and a major part of *STRC*, linked to infertility and deafness, respectively. The mother of the proband has also a homozygous deletion in the region whereas the father is a heterozygote.
For confirmation of the deletion a PCR was performed for an STR marker and no products have been found in the proband and the mother.
The genotypes and the phenotypes of all affected individuals in the family are discussed in detail.
A family with deafness-infertility syndrome presenting in two generations

PM01.32

Recurrent microdeletions at Xq27.3-Xq28 are not associated with infertility of males from the Czech Republic

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Introduction: Genetic causes of male infertility are hypothesized to involve multiple types of mutations, from single gene defects to complex chromosome rearrangements. Recently, several recurrent X-chromosome microdeletions (located in subtelomeric region of the long arm) were reported to be associated with male infertility in Spanish and Italian males (Lo Giacco et al., 2014). The aim of our study was to test their prevalence and infertility association in population of men from the Czech Republic.
Materials and Methods: 146 males with idiopathic nonobstructive infertility were compared to 109 males with normal fecundity. X-chormosome microdeletions were assessed by +/- PCR with three primer pairs for each region Xcnv64 (Xq27.3), Xcnv67 (Xq28) and Xcnv69 (Xq28). The latter microdeletion was also complemented with amplification across the deleted region, dividing the deletion into three types.
Results: We detected presence of Xcnv64 in 10 patients and 12 controls, Xcnv69 in 5 patients and 4 controls (3, 1 and 1 patient vs. 3, 1 and 0 control for types A, B and C respectively). Thus the frequency was comparable in patient and control groups (Fisher´s exact test P>>0.05). The patient with Xcnv69 type C deletion also carries Xcnv64 deletion. This may indicate a more extensive rearrangement with putative causal potential.
Conclusion: Association of X-chromosome microdeletions at Xq27.3 and Xq28 with male infertility could not be confirmed for Czech males. One patient may carry a larger rearrangement that will be further dissected.
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PS01.33

Maternal plasma microbiome detection by analyzing sequencing data of non-invasive prenatal test

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Introduction
MPS-based non-invasive prenatal test not just supply an effective method for detecting chromosome abnormality, but accumulate large amount of sequence data of plasma cell free DNA. Main purpose of this research is unfolding the plasma microbiome through using NIPT sequencing data. A whole scene of exogenous organism in human blood would be presented, together with exploring differences between T21-positive and T21-negtive samples.
Materials and Methods
A cohort of 40,934 plasma samples undergone NIFT test was obtained. Sequencing reads mapped on human reference were filtered out. The remaining non-human reads were assembled to contigs by five different pipelines. Contigs were annotated by Nucleotide database and then classified into different taxonomy groups. Each sample's raw reads were then remapped to the constructed reference contig set. Clustering analysis was performed. Moreover, non-human sequence abundance of trisome 21 samples and normal samples were calculated separately.
Result
15,350 contigs being annotated on nucleotide database constructed a reference set and classified into 16 taxonomic categories. The non-human rate of T21-positive group and T21-negtive group did not show significant difference. However, remap rate of Trisomy 21 samples was lower than that of non-trisomy samples. In addition, cluster analysis showed a remarkable correlation between microbiome abundance distribution and sampling geography locations.
Conclusions
This research built a reference set of plasma microbiome and demonstrated the complexity of plasma microbita. Moreover, differences of the microbiome between T21-positive and T21-negtive samples would supply evidences for enhancing the prenatal test and assisting the detection of some other diseases.

PM01.34

Hsa-let-7c miRNA as a potential biomarker for congenital heart disease

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Background: Congenital heart defects (CHD) are the most common fetal malformations and often correlated with chromosomal abnormalities. There is a great need for biomarkers which could detect CHD early with high accuracy. The miRNAs are short, non-coding RNA molecules that play important role in regulation of eukaryotic gene expression. Let-7c miRNA is located on the human chromosome 21 and broadly expressed by the major types of cardiovascular cells. Recent studies revealed the possible role of let-7c in heart development; therefore we hypothesize that it can be associated with CHD.
Purpose: Based on our previous results, let-7c is significantly upregulated

in the circulation of mothers who has fetuses with CHD. In this study, our aim was to analyze the let-7c expression in diseased fetal heart samples to confirm the miRNA's importance in pathogenesis of CHD.
Patients and methods: We have collected heart samples from fetuses with CHD and/or trisomy 21 and from healthy controls. Total-miRNA was isolated from the left ventricles; the quality was checked by UV-VIS spectrometry. Total-miRNA samples were reverse-transcribed. RT-PCR was performed on the cDNA templates using let-7c specific primers. U6 snRNA was used as control.
Results: We found significant differences in the let-7c concentrations between the control and the diseased fetal heart samples: 0.0021±0.00075 ng/µl vs. 0.031±0.041 ng/µl ($p<0.05$). The highest expression was observed in the cases of trisomy 21 with accompanying CHD. According to our studies elevated let-7c expression is associated with CHD and may be ideally suited as a biomarker for the disease.

PS01.35

The genetic stability of human blastocysts can be effectively predicted by the copy number of mitochochondrial DNA detected by next generation sequencing (NGS)

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Summary answer
Is the copy number of mitochondrial DNA (mtDNA) correlated to the genetic stability and developmental potentiality of blastocysts?
Whole genome amplification of single-cell and next generation sequencing (NGS) can be used to analyze embryo genome and mtDNA.
Study design, Participants/materials, setting, methods
A retrospective study was performed at the Reproductive and Genetic Hospital of CITIC-Xiangya, and BGI-Health, China, involving 440 couples with indications to in vitro fertilization treatment, 1528 blastocysts were biopsied and frozen embryo transplant was carried out using embryos with balanced genome. Embryos were subjected to preimplantation genetic diagnosis/screening using next generation sequencing (NGS-PGD/PGS) between October 2011 and September 2014.
Main results and the role of chance
The sequencing data covered 5.5% ± 1.2% of the whole human genome and 98.7% ±3.1% of mtDNA.The copy number of mtDNA in euploid blastocysts was significantly lower than that of the chromosomally abnormal blastocysts(291.46 vs 317.39,$P<0.001$). Significantly reduced copy number of mtDNA was also found in blastocysts from young women (age≤35 years) comparing to blastocysts from the aged women(300.07 vs 322.07,$P=0.002$). Similarly, The copy number of mtDNA also correlated to the development rate of the embryo and the blastocyst quality, blastocysts with higher ranking contained considerably fewer copies of mtDNA than blastocysts with lower ranking and poor development($P<0.001$).
Limitations
Complete pregnancy outcomes could not be obtained as some blastocysts were not transferred yet. Future data collection is warranted.

PM01.37

Utilization of noninvasive prenatal screening and its relevance to clinical practice: Update on clinical outcome metrics on over 85,000 cases

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Objective: The
verifi® noninvasive prenatal screen (NIPS) has been available through Illumina's accredited clinical lab since February 2012. Professional societies have published statements supporting the use of NIPS and recommend continued test performance monitoring. In follow-up to Illumina's first published clinical experience paper (Futch et al, June 2013), this study highlights continued efforts to provide clinically relevant metrics for chromosomes 21, 18, and 13.
Method: Outcome information (karyotype or birth outcome) was requested from providers for singleton samples reported as aneuploidy detected (AD) or suspected (AS) for chromosomes 21, 18, or 13. Voluntary outcome reporting was encouraged for all discordant outcomes.
Results: Of 86,658 cases, 85,298 (98.4%) met inclusion criteria for NIPS result reporting, 101 (0.1%) were cancelled for technical reasons and 1259 (1.5%) were cancelled for administrative reasons. Average turn-around-time was 3.3 business days. Of 85,298 reported samples, there were 2,142 (2.5%) positive results: 1,858 AD (2.2%) and 284 AS (0.3%); AS results have significantly decreased since 2012. Informative clinical outcomes were available for 851 (39.7%) positive samples. Of 85,298 reported samples, 108 (0.13%) AD cases were reported as putative false positives; 15 (0.02%) false negatives were reported. The observed overall (all chromosomes) positive predictive value was 94.2% for AD samples and 88.9% for AD/AS samples combined. The overall observed negative predictive value was over 99.9%.
Conclusion: Test modifications have facilitated a refinement in borderline result classification, and improvements in turn-around time and cancellation rates.Information about clinical performance of NIPS aids in appropriate pre- and post-test counseling.

PS01.36

Comparison of two academic software (RAPIDR and WISECONDOR) for aneuploidies detection using semiconductor sequencing data in a NIPT process

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Based on a statistical analysis of low coverage genome sequencing data, non-invasive prenatal testing of aneuploidies is being provided in a growing numbers of countries. It has proved a major improvement versus classic screening strategies but still requires invasive procedures when positive. Using five hundred samples included in our French multicenter study, we aim to validate two different published bioinformatics tools for aneuploidy calling. All patients included had an indicated invasive sampling to achieve fetal karyotype in parallel.
WISECONDOR (WIthin SamplE COpy Number aberration DetectOR) and RAPIDR (Reliable Accurate Prenatal non-Invasive Diagnosis R package) both require a reference set of euploid samples. RAPIDR establishes a baseline for confrontation with unknown samples. WISECONDOR uses a "within sample" normalization algorithm made from the reference set and preventing from inter-individual variation. They both use a bin segmentation approach to take into account the GC-content bias. Different QC metrics are used. Fetal fraction, a well-known cause for false negative, can be estimated for male pregnancies via RAPIDR. WISECONDOR uses an inter-chromosomal concordance test to reduce technical noise. A training set of 50 samples has shown comparable performances regarding False Negative and False Positive rates for main aneuploidies (T21, T18, T13). The study will focus on the practicality for use in routine diagnosis (time of calculation, necessary resources, setting up), reliability of QC metrics and potential discrepancies.
Both applications were initially developed using data from Illumina dye sequencing technologies. In this study we will demonstrate that semiconductors sequencing data fit to these two turnkey methods.

PM01.38

False negative NIPT results for trisomy 13, 18 and 21: risk figures derived from cytogenetic investigations in chorionic villi

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Background: Non-invasive prenatal testing (NIPT) demonstrated a small chance for a false positive and false negative result. This is partly due to the fact that the fetal DNA in maternal plasma is derived from the cytotrophoblast of chorionic villi (CV). This cytotrophoblast is not always representative for the fetus because of its embryonic origin (trophoblast) and the existence of chromosomal mosaicism. Therefore, accurate cytogenetic studies in CV involve the investigation of both cytotrophoblast (STC-villi) and mesenchymal core, the latter having the same embryonic origin as the fetus itself. We calculated the risk for a false negative trisomy 13, 18 and 21 NIPT result of a biological nature based on our experience with CV.
Me**thods :** All cases of fetal trisomy 13, 18 and 21 among 5967 CV samples of

pregnancies at high risk that were cytogenetically investigated in our centre between January 2000 and December 2011, were retrospectively studied for the presence of a normal karyotype or mosaicism < 30% in STC-villi.
Results: 404 cases of trisomies 13, 18 and 21 were found amongst 5967 samples. Of these 404 cases, 14 (3,7%) had a normal or low mosaic karyotype in STC-villi and therefore would potentially be missed with NIPT. It involved 2 % (5/242) of all trisomy 21 cases and 7.3% (9/123) of all trisomy 18 cases.
Conclusion: In 1:426 (14/5967) NIPT samples of patients at high risk, a trisomy 18 or 21 will be missed due to the biological phenomenon of absence of the chromosome aberration in the cytotrophoblast.

PS01.39
Identification of 22q11 microdeletions by noninvasive prenatal testing (NIPT) - one year of clinical experience

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Introduction: NIPT for fetal aneuploidies has become routine practice in pregnancy management. A whole genome approach enables detecting subchromosomal events by employing a novel algorithm that uses low coverage sequencing data. The output for detected fetal microdeletion events is akin to data from conventional microarray analysis of invasive testing.
Methods: Maternal blood samples submitted to Sequenom Laboratories were subjected to DNA extraction and library preparation followed by whole genome massively parallel sequencing. Sequencing data were analyzed using an algorithm to detect subchromosomal events such as 22q11 microdeletions. Results: The MaterniT21® PLUS test identified 32 cases with a 22q11 deletion. For twenty-one cases diagnostic invasive testing was performed and for all cases the 22q11 deletion was confirmed. Eleven cases did not have invasive testing results available. Seven of these cases had clinical findings of complex heart defects or Tetralogy of Fallot consistent with the NIPT result. For the remaining four cases, no clinical signs were identified and outcomes were pending. Thus far no confirmed false positives have been identified. One of the confirmed cases was a twin gestation where one twin was positive for the 22q deletion.
Conclusion: It is imperative when testing for rare conditions that tests perform with the utmost specificity to yield high positive predictive values. By using a whole genome sequencing approach, we have demonstrated that this objective is achievable. This abstract provides further evidence to broaden the scope of non-invasive testing to detect subchromosomal deletion/duplication events and the potential to derive fetal karyotypes in the future.

PM01.40
When isochromosomes and noninvasive prenatal testing (NIPT) collide: The technical and clinical challenges of piecing together puzzling cases

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Background: Noninvasive prenatal testing (NIPT) uses circulating cell free DNA for the evaluation of fetal chromosomal abnormalities. Whole genome sequencing combined with advanced bioinformatics enables detailed interrogation of a variety of complex chromosomal changes. Here we highlight three cases involving isochromsomes and relate their NIPT results to diagnostic and clinical outcomes.
Case 1: mos 47,XX,+i(18)(p10)[3]/46,XX[16]
NIPT performed at 12 weeks gestation indicated Trisomy 18. CVS chromosomes revealed a mosaic marker chromosome and microarray detected an 18p duplication. Fetal ultrasound was normal. Amniocentesis chromosomes revealed mosaicism for a supernumerary isochromosome 18p. NIPT traces corroborated duplication of 18p.
Case 2: idic(Y)(q11.2)[15]
NIPT performed at 12 weeks gestation indicated Turner Syndrome. Fetal ultrasound showed male genitalia. Amniocentesis chromosomes and microarray revealed an isodicentric Yp. Discordant NIPT results may suggest the placenta is mostly 45,X, though traces reflect presence of Yp material.
Case 3: 46,X,i(X)(q10)[13]/45,X[7]
NIPT performed at 10 weeks gestation indicated Turner Syndrome. Fetal ultrasound was normal other than a clubbed foot. Amniocentesis chromosomes revealed a mosaic karyotype including an isochromosome Xq. NIPT traces show no evidence of segmental abnormality and may suggest the placenta is mostly 45,X.
Conclusions: Isochromosomes arise from an error in centromere division during meiosis or mitosis. Most are de novo and mosaic in origin, consequently having variable impact on developing embryologic tissues. As NIPT reflects placenta tissue, results may be concordant or discordant with amniocentesis studies. NIPT can lend insight into the timing and origin of such complex events and help explain inconsistencies between testing modalities.

PM01.42
Comparison of NIPT clinical performance in 72,382 high-risk pregnant women and 40,287 low-risk pregnant women

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NIPT has been applied in prenatal screening for fetal aneuploidy with remarkable advances. However, clinical data from large scale of NIPT practice in the general population has not been reported. We prospectively analyze NIPT performance in 147,314 pregnancies with singleton and twins from 508 hospitals from January 1, 2012 to August 31, 2013, which is the largest clinical experience to-date. NIPT sensitivity and specificity were validated by karyotyping confirmation for positive cases and clinical follow-up of negative cases, showing comparable if not better performance comparing to previous studies in small scale of high-risk population in detecting T21, T18, and T13. A performance comparison was also performed between the high-risk group and the low-risk group, which were divided based on maternal age, prenatal screening results, nuchal translucency measurement, family history and previous pregnancy of aneuploidy, showing the equivalent effectiveness of NIPT in the low-risk population as in the high-risk population. In the total population, NIPT false positive and false negative results were investigated for their corresponding reasons. Biological factors such as maternal background and mosaicism were the major reasons causing NIPT false positive and false negative results. Our data supports the use of NIPT in the general population to screen for T21, T18, and T13.

PS01.43
Non-Invasive Prenatal Testing for the most common aneuploidies (trisomies 21, 18, and 13) using a semiconductor-sequencing platform: a French multicenter pilot study

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Combined first-trimester screening has improved prenatal screening for trisomy 21. However the number of unnecessary invasive diagnostic procedures still remains high. Non-Invasive Prenatal Testing (NIPT) using massively parallel sequencing of cell-free fetal DNA from maternal plasma, which is now part of the prenatal landscape, should drastically diminish the risk associated with invasive techniques. Several publications have established NIPT's effectiveness using mainly the Illumina sequencing technology.
A French consortium of seven academic hospitals collaborates to validate a common protocol and to evaluate the efficiency and reliability of NIPT of the most common chromosomal aneuploidies using a semiconductor-sequencing platform. Indeed many French laboratories are already equipped with this technology.
A total of 500 pregnant women (between 12.3 and 35 weeks of gestation) who presented a high risk of aneuploidy and underwent fetal karyotyping were included in a prospective study. 15 % of these patients presented a fetus with one of the most common aneuploidies: trisomies 21, 18 and 13. The NIPT results matched the fetal karyotyping results in all of the cases: all trisomies were detected. The analysis of whole genome sequencing data (including notably librairies quantification, total raw reads per sample, estimate of plasma fetal DNA fraction) enabled us to establish the quality criteria

required for its use in routine diagnosis.
NIPT using a semiconductor-sequencing platform is a rapid and cost-effective alternative technology, and represents an attractive approach for large scale population NIPT.

PM01.44

Validation of abnormal non-invasive prenatal testing (NIPT) by conventional testing technologies - Tel Aviv Medical Center experience

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Accurate assessment of fetal aneuploidy risk is important for genetic counseling in order to facilitate informed reproductive choices while avoiding unnecessary pregnancy loss due to invasive testing. Recent non-invasive prenatal testing (NIPT) using cell free DNA screens for common aneuploidies and some microdeletion syndromes. This technology is offered in Israel since 2012 as an out-of-pocket service. Abnormal results are usually followed-up with invasive testing. We hereby describe our experience with such cases.
During the years 2013-2014, twenty one women underwent invasive testing in our institution due to abnormal NIPT results. These included 10 cases at risk for trisomy 21, 5 for sex chromosome aneuploidy (SCA), 2 for trisomy 13, one for trisomy 18 and 3 for a suspected microdeletion. QF-PCR was performed in 14 of the 18 cases at risk for aneuploidy, all cases underwent full karyotyping. Chromosome microarray analysis (CMA) was performed for cases at risk for microdeletions.
The overall rate of discordant results in our series was 40%. The highest detection rate was for Down syndrome with a positive predictive value (PPV) of 90%. The PPV for SCA was 60% (3 of 5). None of the 3 cases suspected for trisomies 13 and 18 were confirmed by karyotyping. Likewise, none of the 3 cases suspected for microdeletion were detected by CMA.
Validation of abnormal NIPT results by conventional invasive procedures remains the gold standard and is essential for establishing fetal status, also demonstrating the importance of physician education regarding the limitations of NIPT.

PS01.45

Massively Parallel Sequencing (MPS) reliably identifies trisomy 21, 18, and 13 in maternal plasma with low-level fetal cell-free DNA fractions.

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Noninvasive prenatal testing (NIPT) detects common fetal aneuploidies by analyzing cell-free DNA (cfDNA). Current methods use massive parallel sequencing (MPS) or targeted sequencing. Several studies have reported that NIPT accuracy is substantially affected by low-level of fetal fraction (FF), referred as to the fetal component of total (maternal + fetal) cfDNA. Targeted sequencing-based NIPT approaches use a 4% FF cut-off, below this value a redraw is requested. However, data describing the limit of detection (LOD) at low FFs is lacking. Here, we determine the LOD for a MPS-based NIPT.
Serial dilutions were made using 26 confirmed fetal aneuploidy samples with a known FF. Each aneuploidy sample was mixed with a euploid sample to create 6 samples with effective aneuploid FFs of 1-4%. Additionally, NIPT was performed on 1998 pregnancies with confirmed outcomes. Fetal fraction was determined in aneuploid and male samples using MPS tag counting.
Dilution experiments revealed a LOD of 2% for Trisomy (T) 21, and 1.5% for T18 and T13. All (26/26) aneuploidy pregnancy samples were detected; 15.4% (4/26) had a FF of 2-4%, none were <2%. Of 1056 euploid male samples, 23 (2.2%) had a FF <2% and 64 (6.1%) had a FF between 2-4%. All NIPT data were concordant.
Our MPS-based NIPT detected aneuploidies down to 2% FF; 6% of samples had a FF in the 2-4% range. Assay LOD should be determined prior to clinical application, as this establishes an appropriate cut-off that lowers the risk of false negative results and avoids unnecessary test cancellations.

PM01.46

Non invasive prenatal diagnosis (NIPD) of RHD using cell free fetal DNA (cffDNA) from maternal plasma as a method for targeted anti RhD prophylaxis

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Introduction: RhD blood group incompatibility between an RhD-negative mother and an RhD-positive fetus followed by allosensitization and production of maternal anti-D antibodies is still the major reason for hemolytic disease of the fetus and newborn (HDFN). Routine antenatal and postnatal anti-D prophylaxis has reduced the risk of RhD alloimmunization to 0.05-0.4%. However about 10-20% of RhD-negative mothers would receive unnecessary prophylaxis. Non-invasive diagnostic methods employing cell free fetal DNA (cffDNA) from maternal blood overcome the risks associated with invasive procedures used for fetal RHD genotyping.
Materials and Methods: We aimed to evaluate the results from non-invasive fetal RHD typing using cffDNA from maternal plasma in a group of 24 RhD-negative women with confirmed singleton pregnancy before receiving their first prophylactic dose of anti-RhD-IgG. RHD genotyping was done using real-time PCR amplification of exon 5 and 7 of RHD gene.
Results: Of the 24 RhD-negative pregnant women 5 (20.8%) were in the first trimester (7±2 GW) and 19 (79.1%) were in their second trimester (20.4±2.6 GW). 20.8% (5/24) were in their third pregnancy and in the second trimester. Non-invasive fetal-RhD typing showed that 20.8% (5/24) of the fetuses were RHD negative. Three of them were in the second trimester and 2 in the first trimester, but to mothers in their second and third pregnancy.
Conclusions: These results although limited, clearly show that non-invasive fetal-RhD typing using cffDNA from maternal plasma can be used as a method in clinical practice for targeted anti-RhD prophylaxis and improvement of management of RHD fetomaternal incompatibility.

PS01.49

Experiences of high-risk pregnant women who were offered a choice between non-invasive prenatal testing, invasive testing or no follow-up test

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Objective: The TRIDENT study evaluates the implementation of non-invasive prenatal testing (NIPT) in the Dutch healthcare system. Here we report on the preferences and experiences of high-risk pregnant women who were offered a choice between NIPT, invasive testing or no follow-up test.
Methods: A nationwide prospective cohort study among pregnant women at high-risk for fetal aneuploidy because of first-trimester screening results (risk >1:200) or medical history. Questionnaires were completed after counselling (n=1,106, 86% response) and after test-results (n=686, 67% response), at seven (of the eight) prenatal diagnostic centres.
Results: The majority of respondents (92%) preferred NIPT, 5% invasive testing, 2% were unsure, and 1% declined testing. Main reason to prefer NIPT was safety for the child (92%). Of the 60 women preferring invasive testing, 52% did so because of test-accuracy, 25% desired more rapid test-results, 5% because it provides more information, and 18% reported other reasons. Most women (92%) felt that they made a well-informed decision, and 75% reported that this decision was easy to make. Intention to terminate the pregnancy for Down syndrome was lower among women choosing NIPT (58%) vs. invasive testing (87%). Women were highly satisfied with NIPT. However, 64% perceived the waiting time for NIPT results (mean:11 days (range 5-32)) as too long, while 3% in retrospect would have preferred a different follow-up test, mostly to avoid the long waiting time.
Conclusion: Most pregnant women felt they could make an informed decision. The majority prefers NIPT, mainly because it has no miscarriage risk. Reducing turnaround time for test-results is our next challenge in meeting women's needs.

PM01.50

Non-invasive prenatal testing (NIPT) of aneuploidy by means of next-generation sequencing (NGS) in high-risk pregnancies of fetal abnormalities. The first experience in Russia.

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Introduction: General prenatal screening estimates only indirect markers, such as fetal ultrasound and maternal serum biomarkers. Chorionic villus sampling or amniocentesis are highly precision but carry a risk of 1-3% of cases procedure-related miscarriage. Non-invasive prenatal testing is actively put into practice after discovered fetal cell-free DNA in maternal blood. Objective: To evaluate the possibility of using non-invasive prenatal testing of aneuploidy by means of next-generation sequencing (NGS) in high-risk pregnancies of chromosomal fetal abnormalities. Materials and Methods: Chorionic villus sampling or amniocentesis was done on 200 pregnant women at 11-14 (n=165) or 17-20 (n=35) weeks of gestation due to abnormal maternal serum screening, increase in nuchal translucency and advanced maternal age. Pregnant women also donate blood samples before having the invasive test. NIPT samples were analyzed using low-coverage whole-genome sequencing of plasma cell-free DNA. Z-score was used for fetal aneuploidy detection and the results were validated by karyotyping confirmation. Results: Aneuploidies were confirmed in 17 of 17 T21-positive cases, 8 of 8 T18-positive cases, and 1 of 2 T13-positive cases. 2 false negative cases were identified, both T21 cases, and 1 false positive case - T13. The principal factor contributing to NIPT false positive result was placental mosaicism, whereas false negative results were due to low fetal fraction (<4%).
Conclusions: At this time, NIPT may be an option for women classified as high-risk of aneuploidy, especially in pregnant women with advanced age. Additional studies are needed to introduce NIPT into the routine workflow of prenatal care.

PS01.51
Non-invasive prenatal screening plus (NIPS+) for fetal trisomy 2 and trisomy 5 mosaicism cases

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Background: Non-invasive prenatal screening plus (NIPS+) has been proven to be a powerful method for the routinely detection of trisomies 21, 18 and 13. However, few cases have been reported about other rare aneuploidies. Here we describe two cases of fetal trisomy 2 mosaicism and trisomy 5 mosaicism identified by NIPS+.
Methods: We sequenced cell free DNA isolated from maternal plasma obtained at 13 and 22 weeks of gestation from two health pregnant woman with a singleton pregnancy. The sequenced data were mapped to human genome sequence (hg19). Z-scores were calculated for all the 23 pairs of chromosomes.
Results: Z-score increasing significantly of chromosome 2 and 5 were observed in these two cases and the Z-score values were 2.80 and 10, respectively. The results were confirmed by karyotyping of amniotic fluid cells and showed 47,XY,+2 and 47,XY,+5[5]/46,XY[35] mosaicism karyotypes.
Conclusion: This study show NIPS+ can provide information on other rare chromosomal abnormalities. It can provide useful information for the further investigation and practice of NIPS+.

PM01.52
Noninvasive detection of fetal aneuploidies using targeted sequencing of paired homologous regions

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Current clinical laboratory offerings for noninvasive prenatal testing (NIPT) determine the presence or absence of fetal aneuploidies using aligned sequence read counting methods or by leveraging single nucleotide polymorphisms (SNPs). We hypothesized that using the relative abundance of paired homologous regions may enable an alternative way to detect fetal aneuploidies noninvasively. This study describes the evaluation of paired homologous regions for the detection of trisomy 21 (T21) and trisomy 18 (T18). Assays for 1,060 amplification targets were designed to determine fetal aneuploidy status, fetal sex, and the proportion of fetal DNA present in a sample. Circulating cell free (ccf) DNA was extracted from the plasma of pregnant female donors and all target regions were co-amplified in a single reaction. Amplified products were evaluated using massively parallel sequencing (Illumina) and homolog ratios were determined based on the read depth from each homolog. We measured the performance of the developed assay in a blinded set of 480 ccfDNA samples with fetal genotypes orthogonally validated by the MaterniT21® PLUS Laboratory Developed Test. Samples were sequenced at a mean depth of 2.3 million reads per sample with 432 (90%) assayed samples returning a result. Using orthogonal NIPT results as a reference for each individual, we detected 31/31 T21 samples and 14/16 T18 samples. No false positive results were observed. This study introduces a novel NIPT aneuploidy detection approach using targeted sequencing of paired homologous regions and establishes proof of concept for a low-cost, highly scalable method for the identification of selected fetal aneuploidies with performance and non-reportable rates similar to some other published methods.

PS01.53
Discordant results for sex chromosomal aneuploidies from noninvasive prenatal testing

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In the human fetus sex chromosome aneuploidies (SCA) have the same prevalence as the common autosomal aneuploidies 21, 18, 13. Non-invasive prenatal testing (NIPT) of cell-free fetal DNA (cffDNA) for fetal aneuploidy risk assessment has been shown to be both highly sensitive and highly specific for trisomy 21. However, it is less sensitive for trisomies 18, 13 and sex chromosome aneuploidies. Discrepancies between positive NIPT result and fetal karyotype on chorionic villus sampling or amniocentesis may occur. The source of aneuploidy may be due to maternal mosaicism, maternal malignancy, true fetal mosaicism, a demised co-twin, an anembryonic sac, confined placental mosaicism.
We present two cases of a 31-year-old woman, pregnant in 18 gestational weeks and a 34-year-old woman, also pregnant in 18 gestational weeks. Both were referred to our Unit for amniocentesis due to positive NIPT for SCA: risk for monosomy X, for the first patient and risk for Kleinfelter's syndrome for the second. The ultrasound scans were unremarkable.
DNA was extracted from uncultured amniocytes, amplified with commercial QF-PCR kit and analyzed on ABI 3130. Karyotyping was performed on cultured amniocytes using standard protocol.
QF-PCR and cytogenetic analysis showed normal results, SCA were excluded.
Both pregnancies were still ongoing, with no pathological findings on fetal morphology ultrasound scans. Cytogenetic and molecular-genetic analyses are to be done after delivery to reveal the source of the discordant results. We expect maternal mosaicism and confined placental mosaicism to be the main causes.

PM01.54
Noninvasive Prenatal Testing after discovery of fetal malformation by ultrasound: two years of experience in France

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Detection of fetal DNA in maternal blood allows non-invasive prenatal testing (NIPT) of aneuploidy with very high sensitivity and specificity. Using NGS, this approach can be proposed to women with higher age, with history of trisomy, with positive test for aneuploidy or in case of parental balanced translocation involving chromosomes 13-18-21.
From January 2013 to December 2014, 2304 tests were carried out in your unit mainly because of advanced maternal age or abnormal first trimester screening (FTS). For 91 women (3.9%), fetal DNA was proposed while scan detected fetal malformations (28.5%: thick nuchal translucency; 21.4%: soft signs of trisomy 21; 12.85%: intrauterine growth retardation; other: 37.27%). For 2 cases, the result was positive for trisomy 21.
Classically, fetal DNA testing is not a good option in case of ultrasonic malformations. For all these women, we granted the request for specific reasons: refusal of patients to perform an invasive procedure due to the risk of induced miscarriages, risk of premature delivery or history of premature labor, decision to continue the pregnancy whatever the outcome, assisted procreation history. Genetic counseling is mandatory before testing. Test limitations were explained. Ultrasound evaluation in our unit was also performed. Clinical evaluation of newborns and follow-up were always practiced after birth.
In some specific situations, NIPT can be performed after genetic counseling and explanation of its limitations. In the near future, it appears important to evaluate the help of this non-invasive approach, mainly after discovery of soft signs in favor of Down syndrome.

PS01.55

Novel homozygous NLRP7 mutation in a Cypriot patient with recurrent hydatidiform molar pregnancies

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Hydatidiform mole (HM) is an aberrant human pregnancy associated with abnormal embryonic development and has, in the majority of sporadic cases, a multifactorial basis. In Western countries, HM occurs once in every 600 pregnancies, however, this rate has been reported to be higher in other parts of the world including the Middle East. Recurrent hydatidiform molar (RHM) pregnancies are rare and are defined by the occurrence of at least two HM pregnancies in the same patient. Recently, mutations in two different genes namely, NLRP7 and KHDC3L have been identified as a cause of familial RHM pregnancies. We present a 37 year old Cypriot patient with a history of four (three of which were histologically confirmed) HM pregnancies. NLRP7 mutation analysis revealed that the index case was homozygous for the p.Leu820Cysfs*29 (c.2458delC) mutation in exon 7 of this gene. This is a novel mutation, not previously reported in the literature. To our knowledge, this is the first Cypriot case with a history of RHM pregnancies in which a causative maternal-effect in the form of NLRP7 homozygosity has been identified.

PM01.56

First successful story of preimplantation genetic diagnosis for pantothenate kinase-associated neurodegeneration

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Here we report the first successful story of Preimplantation Genetic Diagnosis (PGD) for Pantothenate Kinase-Associated Neurodegeneration (PKAN). We received a referral from Pediatric Neurologist concerning genetic diagnosis and reproductive option for the couples bearing the first child affected by this particular disorder. A 2-year-old Thai boy was born from non-consanguineous parents, developed dystonia and neurological deterioration after the age of 1 and died within 1 year after establishing clinical diagnosis. DNA sequencing of *PANK2* in the patient's leukocytes revealed novel homozygous g.21738G>C, whereas the parents were identified as carriers. Genetic counseling for PGD was performed to the couples and the ethical clearance was done. *In-Vitro* fertilization (IVF) and Intra Cytoplasmic Sperm Injection (ICSI) with PGD was performed. All of embryos were biopsied in the cleavage stage and subsequently performed for whole-genome amplification. Genetic status was diagnosed with the linkage analysis using family-specific short-tandem repeat markers and direct mutation testing using SNaPSHOT Mini-sequencing. The aneuploidy screening was performed by Next-Generation Sequencing-based strategy. There were seven embryos from these couples: two likely affected, three likely carriers, one likely unaffected and one failed in the target genome amplification. Aneuploidy screening was done before making decision of embryo transfer and only one unaffected embryo passed the screening. Thereafter, this embryo was transferred in frozen thawed cycle and the pregnancy was successful. The confirmation was done by amniocentesis, which showed the consistent result to PGD. At 38 weeks of gestational age, a healthy male baby was born.

PS01.57

Algorithm for efficient analysis of perinatal samples: a three-year prospective study

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Objective: To evaluate the performance of a clinical algorithm for direct genetic analysis done on fetal/neonatal tissue.
Methods: Study samples consisted of tissues obtained from miscarriage, stillbirth and neonatal demise during the time period from July 1st, 2011 to June 30th, 2014. QF-PCR analysis was the initial test performed on all specimens followed by chromosomal microarray analysis done on the normal QF-PCR specimens.
Results: A total of 1071 of 1195 submitted specimens were confirmed as of true fetal origin. Of those 1071 informative specimens, 30.8% yielded abnormal results. Of the latter, 57.6% had abnormal QF-PCR and 42.1% had abnormal microarray result. Autosomal trisomies were detected in 61.2%, 7.6% had triploidy, 9.1% had monosomy X, 1.5% had sex-chromosome aneusomies, 5.8% were molar pregnancies and 14.2% had copy number variants (CNV) including microdeletions/microduplications and cryptic unbalanced rearrangements (Table 1). The highest diagnostic yield was observed in the 1st trimester specimens 67.9%, followed by 20.3% in the 2nd and 9.2% in the 3rd trimester. We confirmed that maternal age is correlated with the likelihood of autosomal trisomies but not with triploidy, sex chromosome aneusomies, molar pregnancy, or CNVs.
Conclusion: This algorithm, based on uncultured specimens, has replaced standard cytogenetic analysis method in testing of perinatal samples and resulted in a substantially higher diagnostic yield and improved diagnostic rate. Establishing the cause of a miscarriage and stillbirth has clinical and reproductive implications and results in changes in the management of future pregnancies, such as the decision to undertake preimplantation genetic testing.

PM01.58

Introducing CMA (chromosomal microarray) analysis into the advanced IVF lab

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Introduction It is widely accepted that a main cause for IVF failure is aneuploidity of the embryos. It is also claimed that normal morphokinetics of early embryos, as demonstrated by the EmbryoScope, are correlated with normal chromosomal constitution. Introducing CMA technique to the IVF lab is aimed to contribute a reliable and objective test for the selection of embryo with the best prognosis. This may enable the transfer of a single embryo without decreasing pregnancy rates.
Aim To assess the additive prediction value of CMA for embryo selection, compare to the known morphokinetic parameters.
Material and methods For CMA analysis 21 PGD embryos, that were not suitable for transfer, were re-biopsied on day 5-6. Single cells were subjected to whole genome amplification followed by array hybridization (BlueGenome, 24 sure+) and scanning. CMA results were compared with morphokinetic parameter: PB localization, timing and synchronization of cells division and EmbryScope's score.
Results CMA results of 21 analyzed embryos were divided into 5 groups: normal chromosomal constitution (3), X monosomy (2), unbalanced translocation (6), single trisomy (3) and chaotic chromosomal constitution (7). Two out of 3 normal euploid embryos were graded with very low morphokinetic score and 7 embryos with chaotic chromosomal constitution were graded with various scores.
Conclusion This preliminary study demonstrates that the ploidity of cleavage stage embryos can still be determined only by an invasive CMA method. We suggest that timing and synchrony of cell cycles are probably dissatisfactory morphokinetic parameters for predicting embryo chromosomal constitution; however, other parameters may be better ones.

PS01.59

Clinical utility of blastocyst biopsy and vitrification for Preimplantation Genetic Diagnosis by haplotyping (PGH) and sequencing for Marfan syndrome caused by a de-novo FBN1 mutation.

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Background
PGH for monogenic disease requires prior construction of haplotypes around the gene of interest using appropriate family members with known disease status to assign phase to the haplotypes prior to undergoing IVF to generate embryos for testing. In the absence of affected relatives, mutation-carrying haplotypes cannot be identified.
We have therefore developed a strategy for de novo mutations which assigns phase during the PGD cycle; this strategy is facilitated by our blastocyst biopsy and vitrification programme.
Methods
Grand-parental samples were used to construct haplotypes for each partner, leaving phase to be set during the case. Nine eggs were collected; four blastocysts were biopsied and vitrified. Due to allele drop out, phase can only be accurately set when the mutation is present. Three embryos, arrested at earlier stages with visible cells, were therefore collected to optimise finding the mutation in a sibling embryo. Samples underwent MDA-based whole

genome amplification and tested using linked FBN1 polymorphic markers, in conjunction with DNA sequencing of the de novo maternal c.2719C>T allele.
Results
Of the 7 tested samples, 3 were heterozygous for the mutation; linkage confirmed their presence on the same maternal haplotype. Two of the four biopsied embryos carried the opposite maternal FBN1 haplotype and will be replaced in frozen embryo transfers.
Conclusion
Our blastocyst biopsy and vitrification programme has enabled a novel strategy for PGD for carriers of de novo mutations. Extensive work up on gametes or sacrifice of viable embryos in a cycle, required by previous approaches, has been circumvented.

PM01.60
The first experience with detection of aneuploidy using next-generation sequencing within preimplantation genetic diagnosis

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Due to rapid development of genetic methods evolved in routine check of embryos suitable for transfer after preimplantation genetic diagnosis (PGD) of monogenic diseases, we have introduced the next-generation sequencing (NGS) for detection of aneuploidies. Our aim is to join the both methods together for routine examination and to increase probability to transfer healthy embryos without chromosomal aneuploidies and unaffected alleles of monogenic disease from parents.
The products derived from MDA of the embryos five days of age were subjected to the PGD analysis and sequenced in the next step using Ion Proton Sequencing machine and Ion Fragment Library Kit.
From June 2014 we have analysed twenty one families undergoing the PGD of monogenic diseases with the aneuploidy detection using the NGS sequencing. Till now the total number of biopsied embryos has been sixty-six; forty-three embryos have been concluded as suitable for the transfer after the PGD but this number of embryos was decreased to thirty-one by the reasons of the NGS results. Twelve embryos were eliminated after NGS - : one triploid embryo , one embryo with trisomy of chromosome 21, two embryos with monosomy of chromosome 4, two embryos with monosomy of chromosome 16, two embryos with trisomy of chromosome 16, one embryo with monosomy of chromosome 18 in mosaic form and two chaotic embryos.
We concluded to involve NGS after the PGD during IVF is effective and suitable. Thereby, we are able to decrease risk of abortions after IVF caused by the aneuploidies of the transferred embryos.

PS01.61
Shallow whole genome sequencing is well suited for the detection of chromosomal aberrations in human blastocysts during preimplantation genetic diagnosis

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Introduction: Preimplantation genetic diagnosis (PGD) for chromosomal rearrangements is widely used to avoid transferring embryos with genomic aberrations. Currently, genomic microarrays are predominantly used for the detection of unbalanced structural abnormalities and aneuploidies in embryos from parents at risk. In this study we evaluate whether massive parallel sequencing (MPS) can be used in PGD for detecting chromosomal abnormalities.
Materials and Methods: 15 patients with a balanced structural rearrangement were included in the study: 8 reciprocal translocations, 4 Robertsonian translocations, 2 inversions and one insertional translocation. Whole genome amplification and microarray analysis (24sure+, Illumina) was performed on 47 trophectoderm samples from the cohort. In the current study, low coverage MPS on a Nextseq 500 (Illumina) and Ion Proton (Life Technologies) instrument was performed in parallel for those 47 amplified samples. Aberrations were detected using the QDNAseq algorithm.
Results: An average read count per sample of 11 and 10 million was obtained on the Nextseq 500 and the Ion Proton instrument respectively. In total, 6 normal and 41 abnormal embryos were analysed. All aberrations previously detected with arrayCGH could be readily detected in the MPS data and were correctly identified. The smallest detected abnormality was a 5 Mb deletion/duplication hence equaling or even exceeding the resolution of the routinely used microarrays.
Conclusions: This study demonstrates that MPS on a Nextseq 500 or Ion Proton instrument can be applied for the detection of chromosomal abnormalities in PGD embryos. MPS can serve as a more cost-effective and flexible technology for PGD.

PM01.62
Human placental genome is enriched in somatic genomic rearrangements

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Placenta is a temporary, but an indispensable organ in human pregnancy. Similarly to cancer, it is characterized by highly invasive nature facilitating effective implantation of the embryo and its function to support fetal nutrition, growth and development. Rapid multiplication and invasiveness of cancerous cells is facilitated by rearrangements in their genomes. So far only cancerous tissues have been described with high profile of somatic copy number variations (CNVs).
We hypothesized that similarly to cancer, somatic genomic rearrangements are promoted to support placental function. We report for the first time an extensive load of somatic CNVs, especially duplications, in the human placental genomes across gestation and suggest that this phenomenon may be critical for placental development and function to guarantee the normal progression of pregnancy. Identified placental somatic CNVs were significantly enriched in genes involved in cell adhesion, immunity, development, cell cycle. Overrepresentation of imprinted genes in somatic duplications suggested that amplified gene copies may represent an alternative mechanism to support parent-of-origin specific gene expression. The discovery may have clinical implications as placentas from pregnancy complications exhibited altered CNV profiles. Also, in prenatal testing based on cell-free DNA shed into the maternal circulation by the placenta, extensive placental somatic CNVs and mosaicism may interfere with the reliable detection of fetal CNV profile.
REFERENCE: Kasak et al. Extensive load of somatic CNVs in the human placenta. Sci. Rep. 5, 8342 (2015).
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PS01.63
Investigation of the polygenic genetics of Pre-eclampsia and its relationship with other phenotypes

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Pre-eclampsia (PE) is a potentially life-threatening disorder characterised by hypertension and proteinuria after the 20th week of pregnancy. Although no single-locus PE associations have been replicated or achieved genome-wide statistical significance in PE mothers or offspring ("fetal cases"), we evaluated evidence for shared DNA variants responsible for PE and 6 other disease phenotypes of Wellcome Trust Case-Control Consortium 1 (WTCCC1) by using polygenic score analysis (PGSA) and genome-wide complex trait analysis (GCTA) methods (Nature 460:748-752,2009; AmJHumGenet 88:76-82,2011). For PGSA, GWAS SNPs with p-values below specific cut-points were identified in PE "Discovery" meta-analysis results from combined totals of 3830 maternal or 2650 fetal PE cases and ~47,000 controls from the InterPregGen Consortium, deCODE, and ALSPAC. For GCTA, individual-level SNP GWAS genotypes from InterPregGen (1900 maternal or 1000 fetal UK cases, 5500 UK controls) were tested for correlation with WTCCC1 phenotypes (~2000 UK cases, 3000 UK controls for each disease). Both PGSA and GCTA found significant evidence ($p<0.001$) for shared genetic variants responsible for the WTCCC1 Hypertension phenotype and maternal PE despite strict exclusion of pre-pregnancy hypertensives from our maternal cases. No significant evidence of variant sharing between maternal or fetal PE and any other WTCCC1 phenotype was observed by PGSA or GCTA. GCTA indicates that maternal and fetal PE arise from large numbers of causative variants spread throughout the genome with the heritability contributed by each chromosome being approximately proportional to chromosome length (maternal PE $r^2=0.86$; fetal PE $r^2=0.59$).
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PM01.64

Design of a preconception carrier-screening panel including more than 200 genes associated to recessive and X-linked disorders. Our experience sequencing DNA samples from 48 Spanish healthy semen donors

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Introduction:
Next-generation sequencing (NGS) methods allow genetic testing for many disorders with high fidelity, quick turnaround time and a lower cost.
Materials and Methods:
DNA from 48 healthy semen donors, with previous negative studies for the most prevalent genetic disorders in Spain: Fragile X syndrome type 1, spinal muscular atrophy and cystic fibrosis (32 common mutations).
Design and testing of a NGS gene panel including 203 genes for recessive and X-linked disorders genes (106 complete coding and splicing region analysis, 97 partial analysis). Paired-end sequencing, alignment with BWA, and variant calling using GATK.
Results:
Mean coverage: 308,76X (SD=62,62). Regions with >20X coverage: 99,53% (SD=0,22).
Pathogenic variants (PV) and likely pathogenic variants (LPV) per donor: 2,04 (SD=1,50).
Variants of uncertain clinical significance (VUCS) per donor: 6,69 (SD=2,44).
Thirty nine donors presented at least 1 PV/LPV. All of them presented VUCS.
Most PV/LPV were found in: *CFTR* (12 donors), *CYP21A2* (10), *BTD* (7), *PCCB* (5), *DUOX2* (4), *SLC12A3* (4), *POMGNT1* (4), *PAH* (3).
Most of *CFTR* PV/LPV were low penetrance variants or associated to non-classical phenotype. High rate of *CYP21A2* PV/PPV could be due to co-capture of pseudogene and false positive calls because of the methodology employed. *BTD* variants obtained were associated with mild phenotype, consistent with population allele frequencies described.
Conclusions:
Taking together all these results, some aspects should be considered: the need to fully analyze *CFTR* and to confirm the results with gold-standard methodology for genes with pseudogenes or high homology sequences, whether or not to analyze genes associated to mild phenotypes, and the high number of VUCS obtained.

PS01.65

Contribution of chromosomal abnormalities and genes of the major histocompatibility complex to early pregnancy losses

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Background: Pregnancy losses (PL) in 60% to 80% of the cases are associated with chromosomal abnormalities in embryos or fetuses. Immunologic factors contribute to PL especially at the early stages of gestation. In particular, 45% of cases of the early pregnancy losses (EPL) are accompanied by immunologic intolerance to the fetus. One of the main factors involved in immune responses is the major histocompatibility complex (MHC) encoded by human leukocyte antigen (HLA) gene locus.
Aims: The determination of chromosomal abnormalities in samples from EPL and allelic polymorphism of HLA-DRB1 and DQA1 genes in couples with RM.
Methods: Banding cytogenetic analysis; interphase mFISH analysis with the probe panel for chromosomes 13, 14, 15, 16, 17, 18, 21, 22, X and Y; DNA extraction by salting method, PCR, agarose gel electrophoresis.
Results: Cytogenetic and molecular-cytogenetic investigations of EPL material identified karyotype anomalies in 32.4% of cases with prevalence of autosomal trisomy - 42.65%, triploidy - 30.38% and monosomy X - 19.11%. Complex analysis of frequency and distribution of allelic variants of genes HLA-DRB1 and HLA-DQA1 allowed to establish alleles DRB1*0301, DRB1*1101-1104 and DQA1*0501 to be aggressor alleles in women with RPL. The cumulative homology of allelic polymorphism of more than 50% of HLA-DRB1 and HLA-DQA1 loci between partners increases the risk of RPL by almost four times. Conclusion: Detected chromosome aneuploidies in samples from products of conception and changes in the MHC genes can cause the failure of a couples reproductive function and can lead to an early fetal loss.
This work was partially supported by the West-Ukrainian BioMedical Research Center grant.

PM01.66

The clinical utility of genetic testing of tissues from pregnancy losses: the results of an ACGS audit.

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Since the publication of the Royal College of Obstetricians and Gynaecologists green top guidelines for the investigation and treatment of couples experiencing recurrent miscarriages, many UK regional genetics laboratories have implemented the proposals and have ceased offering parental karyotyping but instead are offering a range of genetic testing strategies for tissues from 3rd and subsequent miscarriages. The range of tests offered includes QF-PCR, subtelomere MLPA, karyotyping and array CGH. Combinations of these tests are also being used to investigate later gestation pregnancy losses and fetal losses where phenotypic anomalies have been noted. The ACGS scientific subcommittee has instigated an audit of the outcomes of these tests with respect to gestational age and the presence or absence of fetal anomalies. The data is being used to examine the clinical utility of this testing in i) providing a likely cause for the pregnancy loss, ii) identifying couples with a balanced rearrangement where there is a risk of recurrence, iii) identifying which couples may benefit from prenatal genetic testing or PGD, and iv) establishing the proportion of cases where there is substantial risk of a future affected live born child. Outcomes have been measured in terms of failure rates, diagnostic yield and the significance of genetic findings to the management of future pregnancies for couples. The evidence to date supports the development of a more targeted and consistent approach to genetic testing of fetal loss.

PS01.67

Early pregnancy loss and polymorphism in xenobiotics detoxification genes

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Pregnancy loss and other pregnancy complication can be connected with environmental and lifestyle risk factor, among which effect of chemical compounds is the strongest. Effects of xenobiotics can be modified by allele variants of xenobiotic detoxification enzymes phase I or II. A total of 71 women with early pregnancy loss and 101 control patients were examined by a case-control methodology. The *Ile462Val CYP1A1*, *Arg47His ADH1B*, *Glu487-Lys ALDH*, *I105V GSTP1* polymorphisms were genotyped by allele-specific polymerase chain reaction. Our data demonstrated that the heterozygous *Glu487Lys ALDH* genotypes rate were higher in the pregnancy loss patients (12.71%) compared to the control group (2.0%). There was no difference between two groups detected for other polymorphisms. However, presence of polymorphic variants of genes of 1st and 2nd detoxification phases can have additive effect and cause multifactorial pathology risk increase. It is shown, that combination of polymorphic variants of *ALDH2* и *GSTP1* genes in genotype results in 5 fold pregnancy loss risk increase. Combination of polymorphic variants of *ALDH2, ADH1B u GSTP1* genes in genotype results in 9 fold pregnancy loss risk increase. The results demonstrated that combination of allele variants of 1st and 2nd detoxification phases in woman genotype increases the risk of early pregnancy loss. This study was supported by the federal assignment № 6.98.2014/K from Russian Ministry of Science and Education.

PM01.68

Blastocyst trophectoderm biopsy and Preimplantation Genetic Diagnosis/Screening for aneuploidy using Array-Comparative Genomic Hybridization: one-year results

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Introduction: Preimplantation Genetic Diagnosis/Screening (PGD/PGS) is the earliest form of prenatal diagnosis and allows identification of genetic abnormalities in embryos produced in vitro, prior to their transfer into the uterus.
The identification and transfer of embryos with normal set of chromosomes allow to increase the implant rate per transfer and significantly reduce the likelihood of miscarriage.
Materials and Methods: Array-CGH is performed on trophectoderm cells. The biopsy of blastocyst during the fifth or sixth day of culture, ensures a remarkable accuracy of the results of the genetic analysis, a significant reduction in the risk of mosaicism, and the absence of impairment in regular embryonic development.
Results: During the last year 690 embryos were processed from ICSI cycles performed on 313 infertile couples. In 11% of cases there weren't clear results due to detection of DNA fragmentation or low concentration of DNA. There was a result in 91% of embryos analyzed. Of these: 63% were found to be normal; 37% were found with chromosomal abnormalities.
The pregnancy rate obtained by the transfer of embryos undergoing PGD, resulted to be free of chromosomal abnormalities, was 14.6% higher, compared to the transfer of embryos without PGD.
Conclusions: The intention of the PGD/PGS is to improve live births rates after IVF treatment. PGD/PGS offers one more chance during IVF treatment to increase the selection of embryos for certain groups of patients, including those with advanced maternal age, repeated failure of IVF cycles, repeated miscarriages in parents with normal karyotype or carriers of balanced translocations.

PS01.69

Premature ovarian failure (POF/POI) and array-comparative genomic hybridization (aCGH)

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Background: One of the frequent reasons of the unsuccessful conception is the premature ovarian failure/primary ovarian insufficiency (POF/POI) that is defined as the loss of functional follicles below the age of 40 years, and the incidence of this abnormality is 0.1% among the 30-40 years age group. Among the genetic causes the most common one involves the X chromosome, as in Turner syndrome, partial X deletion and X-autosome translocations.
Methods: Here we report a case of a woman referred to genetic counselling because of POF. Genetic testing of the 27 year old female patient was carried out due to suspected POF/POI. Molecular and cytogenetic analyses were performed. We evaluated the FMR1 gene analysis using Southern blot technique and Primed PCR. We performed the FISH method and the standard cytogenetic analyses by G-banding also. In order to detect the exact breakpoints, we used a special cytogenetic array ISCA plus CGH array.
Results: We detected a large 67.355 Mb size deletion on the X chromosome at the critical region (ChrX q21.31-q28) which is associated with the POF/POI phenotype. At this region the concerned genes were those ones that the different studies published as POF/POI associated (POF1B; BHLHB9; DACH2; DIAPH2, FMR1; FMR2; XPNPEP2; PGRMC1, CENP1, BCORL1).
Conclusions: We conclude that the karyotyping is definitely helpful in the evaluation of POF patients to identify the non submicroscopic chromosomal rearrangement, and using the array CGH technique we can contribute to the most efficient detection and mapping of exact deletion breakpoints of the deleted Xq region.

PM01.70

The Belgian MicroArray Prenatal (BEMAPRE) database.

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Objectives
Since 2013, samples for prenatal diagnosis in Belgium are analysed by Chromosomal Microarray Analysis. Interpretation of prenatal copy number variants (CNV) remains difficult given the limited phenotypic information. An Ad Hoc Committee tries to resolve uncertain cases based on literature and experiences with similar variants. A Belgian MicroArray Prenatal (BEMAPRE) database studies the association between laboratory, ultrasound and postnatal data.
Method
Our database was customised in consultation with the Centers for Medical Genetics to import, consult and extract genotype-phenotype data. Prenatal cases in which a pathogenic CNV/UV(unclassified variant) >400kb was detected, are imported. Phenotypic data are added postpartum and at the age of 2-3 years. Meta-analysis is performed based on genotype-phenotype data from hundreds of cases.
Results
Reporting policy is determined by classification of CNVs (benign, UV and pathogenic). If UVs have intragenic deletions/duplications in a known gene; are mentioned in literature and/or databases; consist of deletions/duplications covering more than 18 genes or comprise an X-linked gene in a XY fetus, likeliness of pathogenicity is evaluated. In case of strong arguments for pathogenicity, parents are tested. They are reported if parental phenotype is potentially divergent or if de novo. Known pathogenic variants, risk factors with high penetrance or ultrasound anomalies and actionable incidental findings are reported. Since 2013, 7875 arrays were performed; 293 (3.72 %) were reported as pathogenic.
Conclusions
The BEMAPRE database is a source of scientific, clinical and ethical studies; allows easy communication among Belgian genetic centers and will be made available to other scientists. Most recent data are presented.

PS01.71

Chromosomal Microarray (CMA) for prenatal referrals with abnormal ultrasound scan findings: experiences of moving to a frontline NHS diagnostic service in the West Midlands, UK

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Prenatal testing by CMA has been established in the West Midlands Regional Genetics Laboratory (WMRGL) at Birmingham Women's Hospital (BWH) since 2009 via involvement in a local project (243 referrals) and the MRC-funded EACH project (125 referrals), with cytogenetic analysis performed concurrently on all samples tested as a stipulation of project criteria
From October 2014, all patients in the West Midlands presenting with abnormal ultrasound scan (USS) findings and/or an NT measurement of >3.5mm have been offered CMA instead of karyotyping, following exclusion of common aneuploidy by QF-PCR. To date >200 CMA analyses have been performed using the ISCA v2.0 8x60k platform.
Only CNVs interpreted to be clearly linked to USS findings or CNVs which are determined to be clearly pathogenic, irrespective of USS, are reported. Gene content and evidence of overlapping CNVs/syndromes in the literature are the main criteria used to assign pathogenicity classifications. Abnormality rate for reportable anomalies is 9.6%. CNVs considered to be sub-microscopic (likely to be undetected by karyotyping) were observed in 4.9% of referrals, consistent with larger published studies.
Decisions regarding clinical reporting and assignment of pathogenicity remain challenging in the current absence of a National UK consensus and within the fast turn-around-times mandated in the prenatal setting. A local review panel including both Consultant Clinical Scientists and Consultant Clinical Geneticists is therefore convened virtually to support clinical reporting. Several complex cases highlighting the need for this approach will be discussed, as will issues encountered in rolling out array-based testing across the whole West Midlands.

PM01.72

Prenatal detection of a mosaic structurally abnormal chromosome 18

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Prenatal detection of a mosaic structurally abnormal chromosome 18
Prenatal detection of true mosaicism involving an autosomal structural imbalance is a rare occurrence. An amniotic fluid sample was received at 20 weeks gestation following abnormal ultrasound scan findings including bi-

lateral ventriculomegaly, possible neural tube defect and a cardiac anomaly. QF-PCR analysis showed no evidence of aneuploidy for chromosomes 13 or 21. However, the chromosome 18 markers, D18S978 and D18S390, were trisomic and there was marked skewing of D18S391 towards the trisomic range. However, the centomeric marker D18S1002 gave a normal result. Fluorescence in situ hybridisation (FISH) studies using the chromosome 18 centromeric probe showed two signals with no evidence of mosaicism. Together, the QF-PCR and FISH findings were suggestive of a mosaic, structurally abnormal chromosome 18. The QF-PCR result for chromosome 18 was reported as being uninterpretable.
Subsequent cytogenetic analysis identified a mosaic, structurally abnormal chromosome 18 in 26% of cells examined. The complex nature of the imbalance could not be fully characterised by G-banding. FISH studies with a wcp18 showed that no other chromosome was involved in the rearrangement and 18p and 18q subtelomeric probes showed that there was both loss of 18q and gain of 18p material. Array CGH was used to further characterise the abnormality. The parental karyotypes were normal indicating that the abnormality had arisen *de novo* in the fetus. Such cases present counselling dilemmas in terms of the uncertainty in predicting the level and distribution of abnormal cells in the fetus.

PS01.73

Non-invasive versus invasive prenatal diagnosis: What do pregnant couples choose?

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Non-invasive versus invasive prenatal diagnosis: What do pregnant couples choose?
Background: Women at increased risk of common trisomies can opt either for non-invasive prenatal testing (NIPT) or invasive prenatal diagnosis (PND) using a SNP array at 0.5 Mb resolution. Array detects more clinically relevant anomalies, and anomalies with variable penetrance and expression, while NIPT only aims to detect trisomy 13, 18 and 21.
Research questions: What do pregnant couples with an abnormal first trimester screening result choose: NIPT or array? Which motivations do pregnant couples have for this choice?
Method: Pregnant women (N=183) and 59 of their partners participated after they were counselled by an obstetrician. Individually, women and their partners filled out a questionnaire assessing anxiety, ambivalence and informed choice, that was previously designed and published.
Results: Eighty-four percent of the women chose NIPT and 16% chose array. The main reason for choosing NIPT was to prevent the risk of a miscarriage and not wanting to test for 'more than necessary'. Women who opted for an array were inclined to obtain more genetic information to be 'prepared for the future', and to mobilise adequate care if needed. Most mentioned they preferred a quicker test result that was completely certain.
Conclusion: While most participants chose NIPT over invasive PND with array, our study shows that for a significant number of pregnant women and their partners, the opportunity to learn more about the health of their unborn child might outweigh the miscarriage risk of an invasive procedure. It therefore seems justified to keep offering this choice.

PM01.74

Audit of prenatal samples referred for specific molecular tests to the Wessex Regional Genetics Laboratory between 2012 and 2014

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Prenatal testing is a significant component of Molecular Genetic Diagnostic Laboratories because of the wide range of tests required and the rapid reporting time. The WRGL received 86, 80 and 91 prenatal referrals for specific molecular tests in 2012, 2013 and 2014 respectively.
There was a slight excess (55%) of amniotic fluid compared to CVS. The tests most frequently requested were DGV (n=51), sequencing (49 referrals for 34 different genes), UPD (n=39), CF (n=38), and skeletal dysplasia (n=29).
Across all referrals, 49/257 results were abnormal. However, pick up was 0/21 for cases with an apparently de novo mutation in a previous pregnancy, 15/46 for recessive conditions and 21/47 for dominant and X-linked conditions. Of 139 referrals with an "intermediate" risk, 11 were affected: this category mainly comprised abnormal scans (9/101) and inherited Robertsonian translocations (0/32).
Abnormal scans accounted for 42% of all referrals, increasing from 34% in 2012 to 45% in 2014; pick-up rate also increased from 0/29 in 2012 to 2/31 in 2013 and 7/41 in 2014. The most common tests indicated by an abnormal scan were DGV (n=47), skeletal dysplasia (n=26) and CF (n=20). Five DGV referrals were affected, three with skeletal dysplasia and one with BWS. There were no positive CF cases referred with echogenic bowel. AF comprised the majority of abnormal scan referrals (88%) in contrast most cases referred through family history were received as CVS (68%).
Additional referrals from abnormal scans, technological advances and increases in the number of genetic tests available are all likely to affect the future delivery of prenatal genetic testing.

PS01.75

Primary ovarian failure in two women with Xq deletion

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Primary ovarian failure (POF) is a pathology characterized by an absence of the normal ovarian function before the age of 40 years. It can be suspected by amenorrhea or premature menopause. Aetiologies are heterogeneous and chromosomal abnormalities can represent 15 to 20 % of reported causes. Nine critical regions (Premature Ovarian Failure POF 1 to 9) were described among which POF1 and POF2 loci localized on the long arm of chromosome X (respectively Xq26-Xq28 and Xq13.3-Xq21.1). Among candidate genes involved in ovarian development localized in these regions, besides *FMR1* (Xq27.3), we can pinpoint *DIAPH2* (Xq22), *XPNPEP2* (Xq25) and *ZFX* (Xp22.2-p21.3) genes.
Karyotype, DNA microarray and fluorescent *in situ* hybridization were performed in two women presenting POF.
The first patient presented a terminal 14.4 Mb Xq27.2q28 deletion including POF1 with *FMR1* as the principal candidate gene. The second patient presented an interstitial 8,4 Mb Xq23.2q22.1 duplication including *DIAPH2* in POF2 and a terminal 53,5 Mb Xq22.1q28 deletion including POF1. These two rearrangements are the result of an inversion-duplication-deletion mechanism not mediated by non-allelic homologous recombination (NAHR).
The presence of a Xq deletion involving POF1 in these two patients consolidates the role of this region in the occurrence of a POF. Furthermore, no case of inversion-duplication-deletion of the long arm of chromosome X in women presenting POF has been reported in literature. Loss of function of the *DIAPH2* gene has already been described as responsible of POF. Nevertheless, the role of duplications involving POF2 and *DIAPH2* remain to be established.

PM01.76

The modifier gene of *Prokineticin 1* variant in human early pregnancy

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Aim: One common missense variant of PROK1-V67I was suggested to play as a modifier in PROK1-PROKR system of human early pregnancy. To explore the modifier mechanism of PROK1-V67I, we studies for functional comparison in gene expression level and cell functions of V67I and its wild type (WT) in transiently transfected cells.
Material and Methods: We investigated transcript expression of V67I and WT in HTR-8/SV neo and HEK293 cells using quantitative RT-PCR, and protein levels of cell lysate and supernatant of culture medium in HTR-8/SV neo, JAR and Ishikawa and HEK293 cells using ELISA method. Transiently V67I- or WT-transfected HTR-8/SV neo and HEK293 cells were used to evaluate cell proliferation, cell invasion, tubal formation, and intracellular calcium mobilization.
Results: The gene expression level of both transcript and protein were down-regulated in all cell lines, ranging from 20% to 70% compared with WT. The ligand activities of V67I and WT on cell proliferation, cell invasion, calcium influx and tubal formation were of no difference. Both PROK1 allele promoted cell invasion and induced intracellular calcium influx activities, whereas they have no significant effect on cell proliferation, and tubal formation. In conclusion, the common variant of PROK1 (V67I) may play as a modifier in PROK1-PROKR system through down-regulation of PROK1 expression. Our investigation may provide a general modification mechanism for impact on disease severity of PROK1-related pathophysiology.

PS01.77

The Baby Bio Bank: a collection of biological samples and medical data from 2500 families affected by recurrent miscarriage, preterm

birth, intrauterine growth restriction and pre-eclampsia available to researchers internationally

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The Baby Bio Bank is a unique collection of over 55000 biological samples collected from pregnancies complicated with recurrent miscarriage, preterm labour, fetal growth restriction, preeclampsia and uncomplicated pregnancies, available to researchers internationally interested in understanding pregnancy complications. Samples are being taken from the three key members of the family, mother, father and baby, allowing hereditary factors from both parents to be tracked
Mothers and their partners were recruited from antenatal clinics and wards from participating hospitals across London. Blood samples for DNA, serum and plasma, were collected from all consenting participants, plus urine from the mother. On the birth of the baby, we collected placental tissue, membranes, umbilical cord and cord blood. As all the samples are intended for DNA, RNA and protein isolation, they were collected, processed and stored to the highest possible scientific standards. Quality control audits followed by downstream applications such as real-time PCR and sequencing have shown clearly that the samples are of high quality and can be used with confidence by researchers.
The biological specimens have restricted value without clinical information. We include clinical information relating to factors affecting pregnancy such as parental height, weight and relevant medical history such as diabetes, hypertension and smoking. Importantly, we also collect fetal outcome data such as gestational age, birthweight and mode of delivery.
We have successfully recruited over 2500 participants and interested researchers are able to apply to use the samples and data. More information on the Baby Bio Bank is available at http://www.ucl.ac.uk/babybiobank including the Baby Bio Bank protocol and information about the application procedure.

PM01.78
Association study of differentially expressed genes regulatory SNPs and preeclampsia: results of a pilot study in Russia

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Preeclampsia is a pregnancy-specific disorder that leads among the causes of maternal and infant morbidity and mortality worldwide. Prevention, early detection, and specific treatment of preeclampsia are hindered by the fact that the etiology has remained unknown. Current consensus implicates placental and endothelial dysfunction, inflammation and genetics in development of preeclampsia. Our prior genome-wide transcriptional profiling of placental tissue led to a novel set of 63 preeclampsia candidate genes. In this report, we present preliminary study on the role of regulatory sites in some of these genes in the genetic susceptibility to preeclampsia. We analyzed 48 regulatory SNPs (rSNPs) in 23 differentially expressed genes in 519 patients with preeclampsia and 718 women with uncomplicated pregnancies from Russian, Buryat and Yakut populations using MassArray iPLEX (Sequenom). We have detected significant associations for preeclampsia with 15 rSNPs in 11 genes (CORO2A, NDRG1, SASH1, BHLHE40, PLIN2, SYDE1, LHB, HK2, INHA, ZNF175, PPP1R12C). These genes were associated with such biological processes as synthesis and functional activity of hormones, protein binding, ligand-receptor interaction and binding with DNA. Interestingly, only one gene (CORO2A) has been associated with preeclampsia in all three populations. These results demonstrate a significant role of genetic variability of the differentially expressed genes regulatory sites in the formation of susceptibility to pre-eclampsia in different ethnic groups. Nevertheless, the clinical significance of these findings remains to be determined. This work was supported by the Russian Foundation for Basic Research (grant №14-04-01467).

PS01.79
Autosomal gene defects investigation of male infertility in germ cell aplasia cases

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Sertoli-cell-only (SCO) syndrome, also called germ cell aplasia, describes a condition of the testes in which only Sertoli cells line the seminiferous tubules and is diagnosed by testicular biopsy. SCOs is considered as irreversible infertility. SCO syndrome, a histological diagnosis, consists of multifactorial reasons including Y microdeletions, kleinefelter syndrome, cystic fibrosis gene mutations, XYY syndrome, cryptorchidism, radiation, cytotoxic drugs and viral infections. The etiology of the disease is currently unknown on the other hand it is believed that autosomal gene defects could lead to SCOs. The aim of this study is detecting autosomal genetic defects and determining candidate genes in SCOs infertile men. Single nucleotide polymorphism + comparative genomic hybridization microarray technology (SNP+CGH array) was performed on 39 SCOs infertile patients in the study. Array CGH compares the patient's genome against a reference genome and identifies uncover deletions, amplifications, ploidy abnormalities and loss of heterozygosity (LOH). We examined a link between defected spermatogenesis genes and infertility. Detected amplifications and deletions in several genes are namely, SHBG, COL1A1, HOXD9, SYCE1, EMX2, EMX2OS, CATSPER2 and loss of heterozygosity in several genes are namely SPATA gene family (SPATA18, SPATA17, SPATA16, SPATA12, SPATA4, SPATA2), TSSK gene family (TSSK3, TSSK4, TSSK6), DNALI1, DNAH5, DNAH11, SPAG16, SPAG8, DMRT1, DMRT2, FSHR, LHCGR, GNRHR, SPACA1, SPACA3, TSGA10, SMCP, KIT, TCTE3, TEX14, FGF8. Amplifications and deletions were detected on some of the genes who play a role in epigenetic changes. Epigenetic genes (H19, KCNQ1, IGF2, CDKN1C) are expected to be linked with male infertility.

PM01.80
Detection of sex chromosome aneuploidies using quantitative fluorescent PCR

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Background: Aneuploidies are the most frequent chromosomal abnormalities at birth. Autosomal aneuploidies cause serious malformations like trisomy 21, trisomy 18 and trisomy 13. However sex chromosome aneuploidies are causing less severe syndromes. For the detection of these the "gold standard" method is the cytogenetic analysis of fetal cells, karyograms shows all numerical and structural abnormalities, but it takes 2-4 weeks to get the reports. Molecular biological methods were developed to evercome on the long culture time, FISH and quantitative fluorescent PCR were introduced. In this work we show our experience with a commercial kit for the detection of sex chromosome aneuploidies.
Methods: We analysed 20.173 amniotic fluid samples in a period of 2006-2013 in our department. A conventional cytogenetic analysis was performed on the samples. We checked the reliability of quantitative fluorescent PCR and DNA fragment analyis on those samples where sex chromosomal aneuploidy was diagnosed.
Results: From the 20.173 amniotic fluid samples we found 50 samples with sex chromosome aneuploidy. There were 19 samples showing 46, XO, 17 samples with 46, XXY, 9 samples with 47, XXX and 5 samples with 47, XYY karyotypes. The applied quantitative fluorescent PCR and DNA fragment analyses method is suitable to detect all abnormal sex chromosome aneuploidies.
Conclusions: Quantitative fluorescent PCR is a fast and reliable method for detection of sex chromosome aneuploidies.

PS01.81
Clinical outcomes for patients with single-nucleotide polymorphism (SNP)-based noninvasive prenatal testing (NIPT) suggestive of fetal sex chromosome trisomy

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Objective: To determine the clinical outcomes for patients within a general screening population who received an NIPT result indicating a fetal sex chromosome trisomy (SCT).
Method: 51,884 consecutive cases were collected from participating clinics over 10 months. Isolated cell-free DNA was amplified and sequenced at 19,488 SNPs covering chromosomes 13, 18, 21, X, and Y; data was analyzed using a proprietary algorithm. Follow-up information was sought for suspected SCT cases.
Results: 65 (0.1%) clinical samples were identified by NIPT as suggestive of SCTs: XXX, XXY, or XYY. All were low-risk for fetal aneuploidy at other interrogated chromosomes. Mean maternal age was 33.9 years and mean gestational age was 15.3 weeks. Karyotype information was available for 19 cases: 17 true positives (5 XXX, 9 XXY, 3 XYY) and 2 false positives (XXX).

This resulted in an overall positive predictive value (PPV) of 89.5%, with PPVs for XXX, XXY, and XYY of 71.4%, 100%, and 100%, respectively. Of the 46 patients without confirmation, 25 declined invasive testing, and no data is available for the remainder. Information about invasive confirmation decisions was available in 44 cases: 17 (38.6%) had invasive confirmation and 27 (61.4%) declined; two of the cases that declined invasive confirmation had genetic testing at birth. Follow-up is ongoing.
Conclusions: Results of this SNP-based approach for SCTs demonstrate good PPVs in clinical practice. Confirmation rate for these disorders in a clinical setting was limited as invasive testing was pursued in less than half of cases where invasive testing decisions were known.

PM01.82
Why is there a relatively low uptake of prenatal diagnosis for sickle cell disease?

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Background: In the Netherlands, each year 40 to 60 children with sickle cell disease (SCD) are born. The uptake of prenatal diagnosis (PND) for SCD is relatively low: an estimated 5 to 7.5% of couples at risk choose PND. Does this reflect an informed choice? The aim was to explore factors affecting this uptake.
Methods: Factors involved in reproductive decision-making of couples at risk for offspring with SCD were explored by: a) performing semi-structured interviews with 21 parents of a child identified with SCD after symptoms (n=13) or by neonatal screening (n=8) (2009); and b) analysing 15 papers (PubMed;1988-2013).
Results: Factors resulting in refraining from PND for SCD were: having no experience with SCD, not experiencing SCD as severe, fear for miscarriages, keeping the child's diagnosis (and own carrier status) secret, fear for stigmatization, religion, a strong wish to have a child, as well as insufficient knowledge about SCD amongst couples at risk and care providers. Those who considered PND, had experience with disease symptoms, were in early pregnancy (when pregnant) instead of later gestational age, wanted to be prepared before birth, had higher socio-economic status, more often single marital status, and more extensively adopted the Dutch culture.
Conclusion: Lack of understanding about the severity of SCD, perceiving high risks for obstetric complications and cultural aspects resulted in refraining from PND for SCD. Improving knowledge among couples at risk and care providers is important and counselling should be repeated. Future non-invasive testing might influence the uptake for PND for SCD.

PS01.83
Prenatally suspected and after the birth confirmed Simpson-Golabi-Behmel syndrome: familial case

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We present fetal ultrasound, newborn clinical and molecular testing findings in a case of Simpson-Golabi-Behmel syndrome (SGBS) diagnosed prenatally and confirmed after the birth. In the first pregnancy (2009), ultrasound examination at 29 weeks of gestation revealed fetal macrosomia (parameters>99‰), macroglossia, depressed nasal bridge, hypertelorism, nephromegaly, clinodactyly and polyhydramnios.
The differential diagnosis between overgrowth syndromes was performed, but most of these syndromes share the same pathological findings, so it was difficult to differentiate them prenatally. However, after comprehensive literature analysis, we notice specific phenotype sings in mother as suspected mutation carrier (Golabi and Rosen, 1984) and fetal facial 3D evaluation, gender, absence of omphalocele and CNS structural defects enabled to concentrate on SGBS as the most probable diagnosis. Since the molecular genetic testing of GPC3 and GPC4 genes in Lithuania was impossible at that moment, genetic counselling after delivery was recommended.
Child (male) was born at 34 weeks of gestation via s/c. After the birth breathing insufficiency, macrosomia, macrocephaly, broad forehead, hypertelorism, depressed nasal bridge, macrostomia, macroglossia, midline grooves under the lower lip, epicanthus, low-set ears, clinodactyly, brachydactyly, polythelia, cryptorchidism and hypotonia were observed. These symptoms coincided with SGBS diagnosis, so molecular genetic testing of GPC3 gene was performed. Deletion of exons 5-8 in GPC3 gene was detected.
In 2011 the same mother gives birth to a healthy son. The third pregnancy (2012) ended in miscarriage at the 13th week of gestation after CVS. Sonography of fetus showed increase NT (3.1mm), enlarge liver and diaphragmatic hernia. The familial mutation was detected.

PM01.84
Prenatal whole genome SNP array diagnosis: relevance of unexpected abnormal results in pregnancies with and without ultrasound anomalies

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Background: We routinely perform SNP array analysis as a first-tier test for all prenatal indications. Array detects more clinically relevant anomalies including pathogenic aberrations that are not related to the indication. The chance of finding these so-called unexpected diagnoses (UD) is one of the reasons that the use of array in prenatal diagnosis is controversial. We will show the relevance of detecting UDs, based on the nature, prevalence, counseling and outcome of the affected pregnancies.
Methods: In 2010-2014 3,326 patients were referred for prenatal SNP array testing (Illumina): 1,682 pregnancies with and 1,644 without ultrasound anomalies. All cases of UD were discussed in a multidisciplinary team consisting of laboratory specialists and clinical geneticists before disclosure. All patients received pre- and post-test counseling. Psychological help was available if required.
Results: In 1:208 (16/3,326) cases an UD was found. Eleven were severe early-onset untreatable diseases. In 9/11 severe cases (e.g. Duchenne muscular dystrophy, Angelman syndrome) the UD helped the couples in making a decision about the course of their pregnancy. In 1 case the UD (22q11 deletion) was found later in the pregnancy when termination was no longer a possibility. In this case delivery was transferred to a tertiary hospital. No severe late-onset diseases were detected.
Conclusion: We will show that in the great majority the UD was relevant for counseling and pregnancy management. This adds another reason to the known recommendations to use SNP array for all prenatal indications.

PS01.85
Molecular cytogenetic examination of female somatic gonadal cells in cryopreserved ovarian tissues: detection of chromosomal aneuploidy and mosaicism.

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We studied somatic ovary cells (SOCs) by molecular cytogenetic methods since they have an important role in nurturing of oocytes.
DNA from ovarian tissue cells of females in their reproductive age (women 29 +/-0.,8 years) who had their ovarian tissue cryopreserved and consented to research was subjected chromosomal aneuploidy screening by VeriSeq (Illumina), MLPA (Subtelomeric mix, MRC Holland), QF-PCR (Devyser) and arrayCGH examinations (24Sure V3, Illumina).
VeriSeq detected low level mosaicism in 4/39 cases (10.3%) with monosomy of chromosome 19, trisomy 4, 21 (isolated) and combined with trisomies 4, 12. QF-PCR did not confirm trisomy 21 mosaicism revealed by VeriSeq, since it is below the detection limit of QF-PCR. On the other hand, MLPA (subtelomeric mix) revealed duplications/deletions of long and short arms in 5/36 (13.9 %): dupl. chrom. 21p (gene STCH), associated in 2/5 cases with del. chrom. 16p (DECR2), in 2/7 with deletion of chromosome 16q (GAS8), in 1/5 with dupl. chrom. 2p/3p (ACP1), including del. chrom. 13p (PSPC1). All suspective results will be expanded by centromeric mix and verified by a-CGH, including low level mosaicism from the VeriSeq assay.
Although the biological significance of these observations is currently not clear, our pilot study indicates that molecular cytogenetic testing in SOCs could provide additional evidence on the genetic quality of cryopreserved ovaries and thus improve outcomes of assisted reproduction from this tissue. Supported by FNM00064203, CZ.2.16/3.1.00/24022, NF-CZ11-PDP-3-003-2014, LD14073 and IGA NT13770.

PM01.86

Nuclear envelope remodelling during human spermiogenesis involves somatic B-type lamins and a spermatid specific B3 lamin isoform

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The nuclear lamina (NL) is a filamentous protein meshwork, composed essentially of lamins, situated between the inner nuclear membrane and the chromatin. Evidence from rodents suggests a role for the NL during spermiogenesis. The mouse spermatid NL is composed of the ubiquitous lamin B1 and the spermatid-specific lamin B3, an N-terminally truncated isoform of lamin B2. To explore the NL in human spermatids, we used RT-PCR on RNA extracted from spermatozoa (remnants of spermatid transcripts) and immunofluorescence on human testis to reveal a lamin B3 transcript present in human spermatids, and B-type lamins as the only lamins detectable in human spermatids. Unlike the mouse, we detected lamin B2 expression in human spermatids. Like the mouse proteins, we show that human lamin B3, but not lamin B2, induces nuclear deformation, when ectopically expressed in HeLa cells. We detected B-type lamins at the nuclear periphery in human spermatids, except in the region covered by the acrosome, and as spermatids mature the B-type lamins recede towards the flagellum. Only lamin B1 remains detectable on 33-47% of ejaculated spermatozoa. On spermatozoa selected for normal head density, however, this fell to <6%, suggesting that loss of the NL signal may accompany sperm nucleus compaction. We also show that the presence of lamin B2 transcripts is variable in human spermatozoa samples. The similarities between lamin expression during human and rodent spermiogenesis, strengthens evidence that the NL and lamin B3 have conserved functions during the remodelling of the mammalian spermatid nucleus.

PS01.87

Familial cases of severe oligozoospermia associated with a homozygous nonsense mutation in a meiotic gene of unknown function

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Gametogenesis is central to human biology, ensuring the faithful and efficient transmission of genetic and epigenetic information to the next generation. An estimated 10% of couples have difficulty conceiving their own child and in 40% of cases there is evidence of a male cause, most often reduced sperm production. Assisted reproductive techniques provide solutions for many couples, but in most cases the primary cause of infertility, and the consequences for future generations, remain undetermined, with only a handful of causal mutations identified.
We have studied a consanguineous family in which five of the six sons have severe oligozoospermia, 0.05x106 sperm per ml (normal = > 20x106). Genome-wide linkage analysis identified a critical region of homozygosity, containing 134 genes (LOD score Z=3.1 at θ=0.01). Sequencing the entire region revealed a single coding variant that was homozygous in the five oligozoospermic sons, heterozygous in the father and absent from public databases. The variant, p.Glu101Ter, is in a gene of unknown function that we have termed OZF13 (Oligozoospermia factor 13), and is predicted to prevent expression of the protein. We have sequenced OZF13 in 100 infertile men with severe oligozoospermia or azoospermia and identified a unique missense variant affecting a conserved amino acid. In human and mouse, we show that OZF13 predominates in the testis and localises to the cytoplasm of spermatocytes. We conclude that OZF13 is required for efficient progression through meiosis, and we have created a mouse model to study the effects of OZF13 loss on spermatogenesis and gamete quality.

PM01.88

Could teratological counseling be used as an effective method in the prevention of unnecessary pregnancy losses among women exposed to diagnostic radiation

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Women are commonly exposed by radiation in the radiology and nuclear medicine departments being unaware of their pregnancies. In such situations, mothers have often deep concern about possibility of having baby with the congenital anomalies. Moreover, families are usually faced to make the decision for termination. In this study, the risk of the congenital anomalies due to radiation exposure was calculated and the importance of „teratological counseling" was demonsrated to avoid unnecessary pregnancy terminations. 139 pregnant women with the history of radiation exposure were evaluated via medical records, retrospectively. Fetal radiation dose was given between 0.01-10.30 (Med. 0.75±1.59 R) to women exposed to radiation for diagnostic purposes, being unaware of their pregnancy. 65% of those pregnant women were suggested to terminate their pregnancy while others were also adviced that their babies may have congenital abnormalities at some level before admitted for teratological counseling. Only 6 women terminated their pregnancy following teratological counseling. Of remaining 133 pregnants, 3 were terminated for medical purposes while 4 were resulted as spontaneous abortion. Remaining 126 pregnancies lasted as normal birth and none of the babies had abnormalities checked by physical examination. In conclusion, we suggest that teratological counseling is necessary to reduce the anxiety of families and it is an effective method to prevent unnecessary pregnancy terminations.

PS01.89

Comparison of microRNA expression in chorionic tissue of healthy and trisomic fetuses

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Recently, there is an effort to associate specific pregnancy pathologies, especially risk of preeclampsia, with particular miRNAs in plasma, which could serve as biomarkers for the detection of the corresponding pathology and so enable its early diagnosis. Direct miRNA analysis in the trisomic placental tissue is an unexplored topic yet.
The aim of this study was to compare physiological and pathological gravidities at level of placental miRNAs expression.
The chorionic villi samples (CVS) collected for the purposes of invasive prenatal diagnosis between 11^{th}-14^{th} gestational weeks from patients with increased fetal trisomy risk were examined. We supposed the differences in presence and concentration of the specific miRNAs between the group of 16 CVS samples with cytogenetically confirmed fetal trisomy of 21, and 10 samples with normal karyotype. The expression of 381 miRNA pattern (TaqMan® Array Human MicroRNA A Cards) was determined using real-time PCR technology in both groups of samples. The results were then statistically evaluated via ExpressionSuite and qBase software. Mammalian small nuclear RNA U6 in combination with small nucleolar RNA RNU48 was used as endogenous controls for appropriate results normalization.
The nonparametric Mann-Whitney test was applied to the both group's expression data.
The group of 20 miRNAs which were significantly elevated in placental tissue of trisomic pregnancies was determined. The p-values ranged from 0.008 to 0.05. The miRNA which most significantly differed between both groups was miR-542-5p. The current results will be subsequently verified in maternal circulation.
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PM01.90

Azoospermia and trisomy 18p syndrome: a fortuitous association? A patient report and a review of the literature

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Complete, isolated trisomy of the short arm of chromosome 18 is very rare. To date, only 24 cases of trisomy 18p have been reported in the literature, making it difficult to define a potentially associated phenotype. However, the available evidence suggests that few clinical features are shared by these patients: only variable intellectual disability, variable facial dysmorphism and epilepsy are reported in a few patients. Although three inherited cases of trisomy 18p have already been reported, all were of maternal origin.
We report on a patient carrying an isolated complete trisomy 18p translocated to the short arm of chromosome 14 and presenting with facial dysmorphism, mild intellectual disability and non-obstructive azoospermia.
Chromosomal abnormalities are more frequent in infertile men with poor sperm quality than the general population. Both numerical and structural chromosomal aberrations have been already reported within the context of azoospermia. To our knowledge, this is the first patient with trisomy 18p to present a fertility impairment due to totally altered spermatogenesis and azoospermia. Although fertility disorders were not mentioned in the four previous reports of men with trisomy 18p, none of the latter had children.

We suggest that azoospermia is a previously uncharacterized feature of trisomy 18p syndrome. We further hypothesize that two mechanisms could be responsible of the fertility impairment: a meiotic synapsis defect due to the additional 18p arm that blocks meiosis, and/or overexpression of a gene located on the 18p chromosome involved in the normal testicular development.

PS01.91

Case report: male patients with normal phenotype and various Y-chromosome rearrangements

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Introduction
Chromosomal rearrangements of the Y-chromosome are known causes for male infertility. Microdeletions in the azoospermia factor (AZF) regions are found in up to 15% of azoospermic or severely oligospermic men (1).
Material & Methods
Here we present data from four patients with normal phenotype, but abnormal sperm count and infertility. For all patients, we performed conventional karyotyping and revealed various rearrangements affecting the Y-chromosome. For more detailed analysis, we performed diagnostics on either molecular-cytogenetic or molecular genetic level.
Results
Screening of the AZFa, AZFb, and AZFc revealed a partial deletion of AZFb in one patient, a partial deletion of AZFb and AZFc in the second one and in the third one, we revealed a complete deletion of AZFb and AZFc. Here, none of the 36 most common mutations in CFTR-gene were found. These data are in concordance with the previously diagnosed oligozoospermia and azoospermia respectively. Interestingly, we could determine a deletion of the PAR2 in two patients using FISH and additionally a duplication of the PAR1 in one of those. We performed SNParray analysis in two patients to determine the constitution of the Y-chromosome. Analysis of one revealed an unclear deletion pattern in the region Yq11.223-q11.23. The analysis of the other one resulted in a huge deletion on Yq11.221-q12.
Conclusions
We will provide detailed case comparison which shows, that performing additional FISH and SNParrays can help to clarify the aberration status of the Y-chromosome.
Literature:
1. Mc Lachlan RI, Mallidis C, Ma K, Bhasin S & de Kretser DM. Genetic disorders and spermatogenesis. Reprod Fertil Dev. 10(1):97-104. 1998.

PS02.01

SLC24A5 recent albinism gene (OCA6): detection of a homozygous mutation in two patient from French Guiana

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Oculocutaneous albinism (OCA) is an autosomal recessive disorder characterized by hypomelanosis of the skin, hair, and eyes, associated with reduced visual acuity, nystagmus, and photophobia. Recently, two new types of albinism non syndromic have been identified OCA6 (SLC24A5) and OCA7 (C10orf11).(1)
Cases : Two women, aged 22 and 65 years, lived in a village along the Maroni River in South America. They were not from the same family. They had a particular albinism, with similar clinical ophtalmologic characteristics : brown iris, photophobia, reduced visual acuity and nystagmus,but their hair was blond at birth and become darker with age. Their skin was light brown. They were sensitive to the sun exposure and might turn brown. The second patient had a significant pachydermia with age (2).
We analyzed these patients by Next Generation Sequencing technology with a panel of genes involved in syndromic and non syndromic OCA (TYR, OCA2, TYRP1,SLC45A2, GPR143, HPS 1 to 6, SLC24A5, MITF, PAX3, SOX10, EDN3, EDNRB). We found the same homozygous variant in these two patients, c.521G>A/ p.Arg174Lys in exon 5 of SLC24A5 gene. This variant had never been described before in the literature. This variant was not reported in the international database of mutations HGMD. There is no frequency data in ESP, EXAC, dbSNP. The prediction software Polyphen and Mutation taster predicted this variant as probably damaging.
Our finding of a novel homozygous mutation in SLC24A5 in two patients from french Guiana strengthens the importance of screening this gene in OCA (3).
(1) Wei et al., 2013 (2) Montoliu et al. 2014 (3) Morice-Picard et al.,2013

PM02.02

The COL4A4 p.Gly533Asp mutation is prevalent in Czech Romani families with Alport syndrome

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Introduction: Alport syndrome is characterized by progressive hereditary nephritis, hearing loss and ocular anomalies. The disease is caused by *COL4A5, COL4A3* and *COL4A4* gene mutations.
Materials and Methods: Twelve patients with hematuria and sensorineural hearing loss were included, in whom carriership of p.Gly624Asp *COL4A5* mutation (the most frequent one in Czech patients) was excluded. Sequencing of the whole coding sequence of the *COL4A3*, *COL4A4* and *COL4A5* genes was performed using either Sanger sequencing on ABI3130 (1 patient) or the ALPORT MASTR kit (Multiplicom) with subsequent next generation sequencing at Illumina platform (11 patients). MLPA was performed to detect genomic *COL4A5* and *COL4A3* rearrangements. In one patient wholegenome SNP array was performed.
Results: Five of the twelve patients carried c.1598G>A, p.Gly533Asp mutation in *COL4A4* gene in either homozygous (4 patients) or heterozygous (1 patient) state. All the homozygots also carried the same 44 polymorphisms in *COL4A3* or *COL4A4* genes in homozygous state. MLPA excluded deletion of *COL4A3*, thus making deletion of the whole region unprobable. SNP array showed loss of heterozygosity of 250 Mb at various sites of the genome of one patient due to consanguinity, including 48 Mb at 2q32.1 to 2q37.1 region (*COL4A3* and *CO4A4* genes are localised at 2q36.3). All the patients with the mutation were of Romani origin.
Conclusion: The *COL4A4* p.Gly533Asp mutation is prevalent in Czech Alport syndrome patients of Romani origin, consanguinity being responsible for its frequent occurrence in homozygous state.
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PS02.03

Phenotypic and genotypic findings in a large cohort of patients with eye anomalies

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Anophthalmia, microphthalmia and coloboma (AMC) are genetically heterogeneous conditions responsible for ~25% of childhood blindness. We aimed to identify causative genes in 311 AMC subjects recruited through a UK national study. Clinical phenotyping and genetic analysis were performed using various methods: single gene sequencing, MLPA, aCGH, custom panel and exome sequencing. Our cohort was subdivided into phenotypic subgroups for analysis: 56 participants (18%) had bilateral anophthalmia/severe microphthalmia, 37 (12%) had bilateral microphthalmia (+/- coloboma), 175 (56%) had unilateral anophthalmia/microphthalmia (+/- contralateral defects), and 43 (14%) had other phenotypes e.g. isolated colobomas or Peters' Anomaly. Thirty-four of the 56 severe cases received a diagnosis, including deletion/mutations in: SOX2 (15 [27%]), OTX2 (7 [14%]), VSX2 (3 [5.4%]), ALDH1A3 (2 [3.6%]), TFAP2A (2 [3.6%]), FOXE3 (2 [3.6%]), also one each: PAX6, STRA6, BMP7, GJA8. SOX2 (7.4%), OTX2 (3.5%) and FOXE3 (1.6%) were the most common causative genes identified, with VXS2, BMP7, CHD7 and TFAP2A each accounting for 1%. Seventy-two participants who initially received single gene screening proceeded to 187 targeted gene sequencing. Pathogenic variants in known genes were found in 5 (including CHD7 and FOXE3); a further 51 had variants of unknown significance (VUS) in known/candidate genes. Of 15 participants with whole exome analysis; 3 had pathogenic variants in known genes (including PAX6 and BCOR) and 9 had VUS. This study represents the largest reported phenotype-genotype study of patients with eye anomalies. To date, 21.5% of participants have a definitive diagnosis and a further 24.4% have VUS and are undergoing further analysis.

PM02.04

Novel variants in OLFM2 in patients with developmental eye disease

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Microphthalmia, anophthalmia and coloboma are developmental anomalies of the eye and occur in approximately 3 in 10,000 births. They are a heterogeneous group of genetic conditions, with 111 genes identified based on published case reports and genetic databases. We identified a heterozygous de novo deletion of chromosome 19p13.2 affecting a minimum of 46kb spanning regions of Olfactomedin 2 (OLFM2) and collagen, type V, alpha 3 (COL5A3) by array CGH in a patient with bilateral microphthalmia and cloudy vascularised corneas. While COL5A3 has not been implicated in mammalian eye function, Olfm2 is expressed in the retina and ganglion cells during mouse eye development. OLFM2 has also been implicated in open-angle glaucoma and elevated intraocular pressure in a Japanese population. Therefore, we screened the exons and flanking regions of OLFM2 for variants in a cohort of 261 patients with developmental eye anomalies using LightScanner® technology, with subsequent validation by Sanger sequencing. We identified novel mutations in three members of this cohort. These included two variants in the 5' untranslated region (UTR) of OLFM2 and an intronic C>A 20bp 5' of exon 5. Of the 5' UTR variants, one is a 4bp insertion/deletion and the second is a G/C substitution, both occurring approximately 60bp 5' of translation start site, separated by only 4bp and predicted to affect transcription factor binding.

We suggest that OLFM2 is a new gene for eye developmental anomalies, in particular associated with microphthalmia, and should be included when considering eye genes to screen in these conditions.

PS02.05

SF3B2, a novel candidate gene for autosomal dominant retinitis pigmentosa, encodes a component of the U2 small nuclear ribonucleoprotein

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The aim of this study was to identify and functionally characterize a novel candidate gene for autosomal dominant retinitis pigmentosa (adRP). Ten individuals of a Belgian adRP family in which known adRP loci were excluded underwent genome-wide linkage analysis, resulting in two novel candidate loci with a maximum LOD score of 1.7. Whole exome sequencing in two affected individuals (HiSeq, Illumina) revealed a missense variant c.2417A>G p.(Tyr806Cys) in the SF3B2 gene encoding the splicing factor 3b, subunit 2. The Tyr residue is highly conserved, several predictions suggest an effect on protein function. The change is predicted to disrupt a phosphorylation site. The variant co-segregates with adRP and is absent in 300 controls. No additional SF3B2 mutations were found in 472 unrelated adRP patients (ERDC consortium). Ubiquitous expression of SF3B2 was demonstrated in human tissues, including retina and RPE. Localization in perinuclear and nuclear areas was shown in 661W mouse cells. Sf3b2 knockdown in Xenopus was performed using targeted injection of a splicing blocking morpholino (GeneTools), showing gross developmental anomalies affecting the eye.

In conclusion, SF3B2 was identified as a novel candidate gene for adRP. SF3B2 is required for binding of the U2 small nuclear ribonucleoprotein (snRNP) to the branchpoint and is involved in early spliceosome assembly. Protein-protein interactions have been identified between SF3B2, SNRNP200 and PRPF8, two proteins implicated in adRP. So far, of the seven known adRP genes involved in splicing, six encode components of the U4/U6-U5 triple small nuclear ribonucleoprotein (tri-snRNP) complex. Our study potentially involves other components of the spliceosome apart from the tri-snRNP complex in adRP.

PM02.06

Autosomal recessive nonsyndromic hearing loss due to TMPRSS3 mutations in Slovenia

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Introduction: Autosomal recessive nonsyndromic hearing loss (ARNSHL) is usually prelingual, nonprogressive and severe to profound. Up to date it was accounted to mutations in 55 genes (Hereditary Hearing Loss Homepage, http://hereditaryhearingloss.org), where the number is still rising. TMPRSS3 gene mutations account for less than 1 % of autosomal recessive nonsyndromic hearing loss (ARNSHL) in Caucasians. In Slovenians, 26.6 % of congenitally deaf patients and 11 % of progressive hearing loss patients had biallelic GJB2 mutations, where other genetic causes of ARNSHL were not exploited so far.

Material and Methods: Targeted next generation sequencing in the index family with congenitally deaf parents and their son was performed initially, followed by Sanger sequencing of selected TMPRSS3 region in 35 patients with ARNSHL and no mutation identified in GJB2 or GJB6 genes.

Results: Next generation sequencing in the index family revealed that a son and his mother were homozygous for TMPRSS3 c.208delC (p.His70Thrfs*19) variant. Father was digenic compound heterozygote for the same variant and common GJB2 c.35delG variant. Additionally, we identified 3 patients homozygous for TMPRSS3 c.208delC in a cohort of ARNSHL patients.

Conclusions: TMPRSS3 mutations detected in altogether 13,1% of the studied patients seem to be an important cause of ARNSHL in Slovenia resulting in uniform phenotype with profound congenital hearing loss and satisfactory hearing and speech recognition after cochlear implantation.

PS02.07

Early-onset Behr syndrome due to compound heterozygous mutations in OPA1

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Behr syndrome (MIM#210000) is characterized by early-onset optic neuropathy associated with ataxia, spasticity, peripheral neuropathy and developmental delay. Although this disorder is believed to be inherited in an autosomal recessive pattern, it might be heterogeneous encompassing several etiologies. Recently, we reported two brothers affected with adult-onset Behr-like syndrome caused by a heterozygous mutation in the optic atrophy 1 (*OPA1*) gene. Heterozygous mutations in *OPA1*, a gene encoding for a dynamin-related GTPase involved in mitochondrial dynamics and mtDNA maintenance, are the main causes of autosomal dominant optic atrophy (DOA). As many as 20% of persons carrying heterozygous OPA1 mutations are affected with the so-called 'DOA plus' consisting in optic neuropathy associated with extra-ocular signs including deafness, ataxia, peripheral neuropathy and mitochondrial myopathy with multiple mtDNA deletions.

We here report four children affected with early-onset Behr syndrome caused by compound heterozygous *OPA1* mutations. These four children are affected with a strikingly similar early-onset neurological syndrome associating severe optic neuropathy (4/4), cerebellar ataxia with cerebellar atrophy at MRI (4/4), peripheral neuropathy (4/4), digestive involvement (2/4) and deafness (1/4).

To date, compound heterozygosity in OPA1 has been proven in 6 patients (including those reported here) all affected with severe early-onset syndromic optic atrophy.

Intriguingly, the same variant p.Ile382Met, involving a highly conserved residue in the OPA1 GTPase domain, was recurrently found in five of six patients in this series. Although the p.Ile382Met mutation on its own might have only mild consequences, it may combine with another mutation to induce a severe pathological condition which is consistent with a semi-dominant mode of inheritance.

PS02.09

Novel gene identified by exome sequencing analysis in a Colombian family affected by autosomal recessive congenital cataract

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Congenital cataract is a Mendelian disorder that affects the eye lens, a non-vascular transparent tissue, causing distortions of the light giving an opaque appearance and loss of transparency that leads to vision problems. This condition is the worldwide leading cause of visual impairment and blindness for children (Chan, Wai H. et al. 2012). Several genes are known to be responsible for the autosomic dominant type of cataract, the most common in the world population. However recessive inheritance has also been reported and it is usually associated with other ocular conditions that worsen the vision making it difficult to treat (Kondo, Y., et al. 2013). In this study, a family of healthy parents and three affected children with zonular, nuclear congenital cataract (two sons and one daughter) is described. Exome sequencing using SureSelect Human All Exon 50Mb V4 (50X depth) was performed to uncover the genes responsible for the condition in the family. Bioinformatic analysis using the reference genome GRCh37 did not find variants in any of the known genes, however we did identify a novel homozygous variant P.Gly377fs in exon 7 of the SLC37A4 gene (NM_001164280.1). According to polyphen (Adzhubei IA. et al. 2010) this variant is considered damaging. Mutations in this gene have been found in patients with glycogen storage disease, however the individuals in our family show no clinical manifestations of the disease. We might speculate that an abnormal glucose homeostasis caused by this mutation might lead to an accumulation of sorbitol forming a sugar cataract.

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PS02.11

Characterization and mutational spectrum of 33 choroideremia families

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Choroideremia (CHM) is an X-linked chorioretinal dystrophy affecting 1 in 50,000 people. Typically, males develop initial symptoms from night blindness to peripheral visual field loss leading to complete blindness. Female carriers are generally asymptomatic, although mild symptoms can be observed. It is caused by mutations in the *CHM* gene that encodes Rab escort protein 1 (REP-1), an essential component of Rab geranylgeranyl transferase (Rab GGTase) that controls cellular trafficking in secretory and vesicular pathways.

We characterized 33 out of 45 unrelated families with clinical diagnoses of CHM. The molecular diagnostic pipeline followed include haplotype analysis, direct sequencing, RNA studies, combined MLPA and qF-PCR, CGH arrays, cytogenetic studies and, in a family, whole exome sequencing. The mutational spectrum included complete or partial deletions of *CHM* gene being the most frequent mutations identified (13 families; 39%). Among them, exon 9 deletion was recurrent affecting three families (10%). Secondly, most pathogenic alleles carried nonsense mutations (11 families; 33%). Mutation p.Arg293* was the most frequent one, being present in four families (12%) from diverse geographical origins: Spanish, Portuguese and Polish. Frameshift p.Thr175fs* mutation was found in one Spanish and one Portuguese families.

Our diagnostic pipeline allowed us to achieve the molecular characterization in 73% of cases. The remainder cases should be clinically reviewed. Alternatively, other mutations in uncovered regions or in other genes can be the cause. To identify the causative mutations in CHM patients is essential in patient management and to the improvement of therapies focused on correcting the primary genetic defect.

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PM02.12

Mutations in Collagen, type XVII, alpha 1 (COL17A1) cause Epithelial Recurrent Erosion Dystrophy (ERED)

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Epithelial recurrent erosion dystrophy (ERED) is an autosomal dominant condition associated with painful corneal epithelial erosions, typically starting within the first decade of life. Between attacks the corneal epithelium either appears normal or there are a small number of epithelial micro-cysts, consistent with a healing epithelial erosion. Sub-epithelial haze may then progress over several decades, which can lead to central corneal scaring. Our goal was to identify the genetic cause of ERED. A large, genealogically expanded, ERED pedigree of northern Swedish origin was identified and whole exome sequencing (WES) was performed using DNA from disparate branches of the family. A novel, non-synonymous COL17A1 variant, c.2816C>T, p.(Thr939Ile), was found to co-segregate with disease in the extended pedigree. COL17A1 encodes collagen type XVII alpha 1, a structural component of hemidesmosomes (multi-protein complexes that provides attachment for basal epithelial cells to the underlying basement membrane) and thus represents an excellent candidate gene for ERED. Previously, in a family with a similar ERED-like phenotype, disease was shown to be linked to Chr10q23-q24 encompassing COL17A1, but the authors reported that no mutation was identified (Sullivan, et al., 2003). Our re-evaluation of this data suggested that the synonymous COL17A1 variant, c.3156C>T p.(Gly1052Gly), that co-segregated with disease could create a cryptic splice donor site. We experimentally confirmed that this synonymous variant leads to aberrant pre-mRNA splicing of the COL17A1 transcript in vitro, and we suggest that both of these ERED-like corneal dystrophies are allelic and can be attributed to mutations in COL17A1.

PS02.13

STX3: A novel gene linked to autosomal recessive congenital cataract, intellectual disability phenotype in a consanguineous Tunisian family

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Introduction

One-third of congenital cataract cases have a genetic cause. Today about 39 loci are involved in isolated or primary cataracts; several complex syndromes including cataract with multiple anomalies and intellectual disability have been mapped. Here is the first report of gene identification in inherited congenital cataracts associated only with intellectual disability.

Materials and Methods

A total of 3 affected patients and 2 unaffected parents belonging to a consanguineous Tunisian family with congenital cataract associated to intellectual disability were analysed.

Homozygosity mapping, a successful method in identifying genetic defects in consanguineous families, we carried out a genome wide scan for the five members.

Moreover, we used the integrated Systems Tool for Eye gene discovery (iSyTE) for the identification of lens specific genes with high or low expression.

Results

GWS identified 2 homozygous candidate regions at chromosome 11 (p11.2-p11.12) and (q11-q13.1). These regions were analysed by the iSyTE allowing the identification of less-highly expressed lens disease-associated genes to non-syndromic congenital cataract. Four genes (STX3 (11q12.1), CCDC86 (11q12.2), SLC3A2 (11q12.2) and SLC15A3 (11q13)) were selected according to their early expression in lens development. All exons, exon-intron junctions were sequenced. By analyzing the results we identified a novel missense mutation c.122A>G in STX3 gene which results in p.E41G. Bioinformatics analysis suggested a deleterious effect of this mutation on protein structure and function.

Conclusion

We report for the first time a missense mutation of a novel lens specific gene STX3 in a phenotype associating AR congenital cataract and intellectual disability. This study highlighted the genetic heterogeneity of congenital cataract.

PM02.14

Application of custom-designed high resolution CGH array in diagnosis of patients with congenital eye malformations

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Introduction: Congenital eye malformations are very highly heterogeneous conditions displaying a wide spectrum of overlapping phenotypes that can occur isolated or taking part of a syndrome. They include pan-ophthalmic disorders such as anophthalmia, microphthalmia (A/M) and aniridia, and also anomalies restricted only to the anterior or posterior segment. Our aim was to design and validate a customized high-resolution CGH array to search for rearrangements across eye developmental genes causing ocular malformations.
Material and Methods: A custom whole-genome oligonucleotide CGH array (Agilent, 4x180K format) was designed to cover all exonic, intronic and regulatory regions from 160 eye developmental genes and the WAGR-related locus at 11p13, using 130K probes. In addition, 44K thousand backbone probes were added to cover the remained non-target genomic regions.
Forty-eight patients with several phenotypes were analysed, including syndromic and isolated forms of A/M, aniridia, coloboma, anterior segment dysgenesis and nerve optic hypoplasia that mostly were previously tested by MLPA for PAX6 or SOX2 deletions.
Results: In 4 patients, our custom aCGH allowed detect larger 11p13 deletions than the previously found by MLPA and then, refined chromosomal breakpoints. In a syndromic patient with bilateral anophthalmia, a 14q22.3-q23.2 microdeletion was detected encompassing OTX2 and SIX6, both genes associated with A/M and septo-optic dysplasia. Non-polymorphic copy number changes were also found at several candidate chromosomal regions. Rearrangements ranged from 6.2Mb to 55kb.
Conclusions: Our custom aCGH represents an accurate tool for the analysis of genomic rearrangements in eye developmental diseases.
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PS02.15

Spectrum of mutations and genotype-phenotype correlation in TGFBI-associated corneal dystrophies

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Autosomal dominant gain-of-function mutations in *TGFBI* are responsible for a diverse range of corneal dystrophies including Reis-Bücklers (RBCD), Thiel-Behnke (TBCD), granular (GCD) and lattice corneal dystrophies (LCD). Here we investigated the spectrum of *TGFBI* mutations in patients with a corneal dystrophy attending Moorfields Eye Hospital. In total, 58 unrelated probands with a suspected *TGFBI*-associated corneal phenotype were screened for mutations by direct sequencing. The most commonly identified mutations occurred at two known mutation hotspot residues of the TGFBI protein, Arg-124 and Arg-555. The mutations included c.370C>T; p.(Arg124Cys) in 17 probands, c.1664G>A; p.(Arg555Gln) in 16, c.1663C>T; p.(Arg555Trp) in 11 and c.371G>A; p.(Arg124His) in 5 patients. The phenotypes associated with each mutation were LCD, TBCD, GCD1 and GCD2, respectively. However, the hotspot mutation c.371G>T; p.(Arg124Leu) associated with an RBCD phenotype was only observed in one proband. Interestingly, the c.1868G>A; p.(Gly623Asp) mutation was identified in 4 patients, with clinical features of RBCD, LCD or both, indicating a lack of genotype-phenotype correlation. Two probands with LCD had c.1859C>A; p.(Ala620Asp) and c.1877A>G; p.(His626Arg) mutations and one proband with RBCD had a c.1874T>A; p.(Val625Asp) mutation. One patient with LCD, who was mutation negative for *TGFBI*, had a mutation in *GSN* that resulted in a re-diagnosis to Meretoja syndrome. In summary, a limited spectrum of *TGFBI* mutations is responsible for the majority of *TGFBI*-associated corneal dystrophies. Mutations affecting residues Arg-124 and Arg-555 show strong genotype-phenotype correlation, whereas mutations affecting other residues show more variable phenotypic expression.

PM02.16

Identification of deafness genes in Israeli Jewish and Brazilian families using Next Generation Sequencing platforms

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Introduction: One in every 500 individuals presents severe to profound sensorineural hearing loss. According to the World Health Organization, hearing loss is the most common sensory impairment. It is estimated that 60% of congenital hearing loss may be due to genetic mutations, with high genetic heterogeneity. Over 80 genes are known to be involved in hearing loss. Traditional diagnostic techniques have become slow and costly for screening this large number of genes. High-throughput platforms based on Next Generation Sequencing (NGS) have provided an optimal solution for diagnostics.
Materials and Methods: We employed Targeted Genomic Enrichment (TGE) and massively parallel sequencing to capture the exons of 284 genes associated with deafness in humans and mice. We performed whole exome sequencing (WES) on a portion of the families. A bioinformatics workflow was implemented, providing an output of potential causative variants. The segregation of a subset of variants was examined in families and controls. When relevant, functional analysis was performed.
Results: One Brazilian and 67 Israeli Jewish families were evaluated. Variants in over 20 genes, including TMC1, POU3F4, MYO6, and MYO15A, were identified, solving 38% of the cases in the Jewish Israeli population. A MYH9 mutation segregated with deafness in the Brazilian family.
Conclusions: These results demonstrate that diagnostics for deafness can be performed optimally using NGS, leading to a better understanding of the genetic profile of hearing impaired populations and mechanisms of hearing loss.
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PS02.17

A genome-wide association study provides evidence of sex-specific involvement of Chr1p35.1 (ZSCAN20-TLR12P) and Chr8p23.1 (HMGB1P46) with diabetic neuropathic pain

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Neuropathic pain is defined as pain arising as a direct consequence of a lesion or a disease affecting the somatosensory system and it affects around 1 in 4 diabetic patients in the UK.The purpose of this genome-wide association study was to identify genetic contributors to diabetic neuropathic pain.
We obtained a GWAS dataset of 6,927 diabetic individuals from the Genetics of Diabetes Audit and Research Tayside (GoDARTS) project and accessed the prescription history of these individuals. Cases of neuropathic pain were defined as diabetic patients with a multiple prescription history of at least one of five drugs specifically indicated for the treatment of neuropathic pain. Controls were diabetic individuals who were not prescribed any of these drugs, nor amitriptyline, carbamazepine, or nortriptyline. Those who had one use of the five specific neuropathic pain drugs were further excluded from controls. Logistic regression analyses were performed to test genetic associations, adjusting with covariates.
Overall, 961 diabetic neuropathic pain cases and 3,260 diabetic controls were identified. We found a cluster in the Chr1p35.1 (ZSCAN20-TLR12P) with a lowest P value of 2.74 x 10-7 at rs71647933 in females and a cluster in the Chr8p23.1, next to HMGB1P46 with a lowest P value of 8.02x 10-7 at rs6986153 in males. The narrow-sense heritability of neuropathic pain from the overall dataset was 14.7%. Sex-specific narrow sense heritability was higher in males (30.0%) than in females (14.7%).
This GWAS on diabetic neuropathic pain provides evidence for the sex-specific involvement of Chr1p35.1 (ZSCAN20-TLR12P) and Chr8p23.1 (HMGB1P46) with the disorder, indicating the need for of further research.

PM02.18

Comprehensive evaluation of the FBN1, LTBP2 and ADAMTSL4 genes in 207 patients with ectopia lentis

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Ectopia lentis (EL) can occur isolated or within a syndromal constellation. The most prevalent cause of congenital EL is Marfan Syndrome (MFS), which requires a lifelong cardiovascular follow-up. Because of the evolving phenotype, FBN1 testing is often requested in children with EL. In patients without an FBN1 mutation, the absence of a definite diagnosis may cause psychological distress and unnecessary examinations. Recently, mutations in LTBP2 and ADAMTSL4 have been implicated in congenital glaucoma and microspherophakia with EL, and EL et pupillae, respectively.
We analyzed the FBN1, ADAMTSL4 and LTBP2 genes in 207 probands referred for EL over a period of 22 months using a step-wise PCR-based next-generation sequencing approach. One hundred fifty-four probands harbored an FBN1 mutation (of which 47 fulfilled the 2010 Ghent criteria for MFS) and 53 did not (of which 9 patients fulfilled the criteria). Of these 53 patients, four harbored biallelic mutations in ADAMTSL4 (c.767_786del; c.2237G>A, c.2021_2022delCT, c.2977C>T, c.963dup). One proband had a homozygous LTBP2 mutation (c.4964A>G; p.Tyr1655Cys). In another proband only one heterozygous LTBP2 mutation (c.3850C>T, p.Arg1284Cys) was found.
FBN1 is the primary gene to screen in EL (mutation detection rate 75%) but if negative should be followed to include ADAMTSL4 and LTBP2 as this reveals a final diagnosis for 11% of the remaining patients (and even up to 15% if the MFS criteria are not met). Our prospective data further indicate that a FBN1 mutation is found in 92.2% of all patients fulfilling the 2010 Ghent criteria for MFS.
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PS02.19

Clinical utility of targeted Nextgen sequencing and array CGH panels along with mitochondrial genome analysis for inherited eye diseases

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Introduction: Next generation sequencing (NGS) panels are increasingly used in molecular diagnosis of genetically heterogeneous eye diseases. We report the yield of these tests in a clinical setting.
Materials and methods: We custom designed a comprehensive panel that included NGS and array comparative genome hybridization (aCGH) for 208 nuclear genes and mitochondrial genome sequencing. We also offered targeted nextgen panels for specific conditions such as retinitis pigmentosa. 222 individuals with eye diseases were tested using comprehensive or targeted nextgen sequencing, mitochondrial genome testing and/or array CGH panels.
Results: We found pathogenic variants in 71/222 (32%) individuals. Of these positive results, 59 were identified by comprehensive or targeted nextgen panels alone, 12 individuals had pathogenic copy number variants identified on aCGH, of which 6 were for dominant conditions and 6 for a second recessive change. In 10 of these individuals, one definitive pathogenic variant was identified, the second was a variant of unknown significance. Another 14 individuals had variants that need additional analysis to determine significance. No pathogenic mitochondrial variants related to eye disorders were found in 99 individuals that had testing. .
Conclusions: Nextgen sequence analysis alone identified ~27% conclusive pathogenic molecular finding and when complemented with aCGH identified an additional 7-8% contributory disease-causing variation but none from the mitochondrial genome sequence analysis. Therefore, complete mitochondrial genome sequencing should be reserved only for individuals with suspected syndromic mitochondrial disorder. The most efficient and cost effective testing is a clinical phenotype driven targeted Nextgen sequencing panel with reflex to aCGH in those that have negative sequencing results.

PS02.21

Genetic and molecular analysis of the GJB2 gene in Moroccan population with non syndromic hearing loss

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Non syndromic sensorineural hearing impairment is inherited in a predominantly autosomal recessive manner in up to 70% of cases. The gene more often involved is GJB2, encoding the gap junction protein Connexin 26. To date, no clinical characterization of the *D F N B 1* inner-ear defects has been reported in our population, which precludes the provision of prognostic information and genetic counseling.
The aim of our study was to determine the prevalence and spectrum of GJB2 mutations, in Moroccan patients and estimate the carrier frequency of the 35delG mutation in the general population.
Genomic DNA was isolated from 60 families/unrelated patients with recessive or sporadic deafness. Molecular studies were performed using PCR and direct sequencing to screen for GJB2 mutations.
Of the 34 cases of family deafness, 9 patients had the 35delG homozygous mutation with a frequency of 26.47%. In sporadic cases the frequency was 15,4 %. We also identified two other mutations: G200R and G59R.
Our data suggest that *GJB2* mutations are the leading cause of moderate-to-profound congenital inherited deafness in Moroccan population. The absence of genetic alterations in the other cases (78.34%) clearly suggests the involvement of other genes that require further genetic analysis.

PM02.22

Multi-Trait Genome-wide Association Studies (GWAS) to investigate hearing function and interactions with BMI and blood pressure

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The genetic bases of complex traits such as Normal Hearing Function (NHF) and Age-Related Hearing Loss (ARHL) are largely unknown. Multivariate GWAS is a new and powerful tool for detecting candidate genes. Here, it was applied to simultaneously analyse hearing thresholds and also combine them with Body Mass Index (BMI) and Blood Pressure (BP), as potentially interacting traits.
A meta-analysis of 2059 subjects from isolated cohorts of Italy and Central Asia were analysed by multivariate linear mixed model regression (Zhou&Stephens 2014) and results were combined, based on inverse-variance weights (R-package MultiMeta). After adjusting for sex, age and relatedness, the following traits were tested: (a) 4 HT (0.5, 2, 4, 8 kHz); (b) HT and BMI and (c) HT and BP.
Preliminary results for HT (a) identified the following top SNPs: rs181948008 (p=4e-09) within *CYP4B1* gene, rs1523730 (p=3.04E-07) close to *CNTNAP2* a gene located within the deafness locus DFNB13 and rs72984055 (p=2.27E-07) within *MMP20* (other metalloproteinases were already involved in hearing loss). In order to investigate previous epidemiological data in which higher BMI was associated with poorer hearing (Curhan et al. 2013), a genome-wide analysis (b) was performed revealing suggestive association with rs62418085 (p=2.47E-07) located within *PRIM2*, a gene already associated with fatty acid levels. Up-to-date results will be presented and discussed.
In conclusion, the multivariate approach can give significant boost in statistical power for correlated traits and allows investigating genetic bases for different related traits.

PS02.23

New mutation found within OTOR gene involved in deafness in two Sudanese families from Al-Jazirah state- Sudan: using Next Generation Sequencing (NGS)

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The next generation sequencing technology (NGS) is one of most promising and cost effective alternative for whole genome exome sequencing, which was used to identify unknown mutations among hearing loss patients. New and possibly pathogenic mutations were screened using NGS (target genes sequencing) for hearing impairment panel including more than 80 genes. Knowing the fact of high rates of false positive pathogen predictions of

found SNPs, different prediction models were combined to enhance prediction power. Two female patients within two Sudanese families from Al-Jazirah state, displayed hearing loss, and Non-synonymous variant were detected and the SNP caused an amino acid substitution in the protein encoded by gene OTOR (OMIM #606067). Then, bioinformatics tools were used to support the significance of this mutation, and also confirmed the genotype-phenotype co-segregation in family members, in addition searched for the SNP that cause mutation within a control exons target SNPs (not founded). The involvement of OTOR in hereditary hearing impairment has not been observed in the Sudanese population so far.

Keywords: Hearing impairment, Next Generation Sequencing, single nucleotide polymorphisms (SNPs), Non-synonymous variant, OTOR gene, Sudanese families

PM02.24

Gene panel testing in non-syndromic hearing loss: validation on a British Asian cohort

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DFNB1, effectively the connexin 26 gene *GJB2,* is the leading genetic cause of non-syndromic hearing loss and testing is integrated in mainstream clinical pathways. However, the list of deafness genes is long - and still expanding - while the *DFNB1* detection rate in most diagnostic streams is in the range of 10%-20%; it can be as low as 5% in British Asians. We explored the possibility of gene panel testing using a custom-designed HaloPlex targeted genomic capture kit (Agilent Technologies) to obtain the coding regions of the majority of non-syndromic deafness genes: 67 genes were included, amounting to 270 kb, with design coverage of over 97%. For validation we sequenced 59 anonymised samples of local British Asian children with suspected genetic hearing loss and no causative *DFNB1* changes through an established pipeline of massively parallel sequencing on Illumina instruments. With structured data filtering and conservative variant evaluation criteria we identified pathogenic or likely pathogenic mutations, verified by Sanger sequencing, in 15 cases within *GIPC3*, *LHFPL5*, *LOXHD1*, *MYO15A*, *MYO3A*, *OTOF*, *PCDH15*, *STRC*, and *TMIE*. Actual average aggregate coverage exceeded 90% at a depth of 30x. The dataset enabled definition of the analytical and clinical scope of the workflow for service consideration, predicting a 6-fold increase in yield compared to *DFNB1* testing, while at the same time sampling the mutation background of non-*DFNB1* deafness in British Asians.

PS02.25

Etiology of hearing loss in a Newborn Screening Program

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Introduction: The Newborn Hearing Screening Program (NHSP) is a way to promote early diagnosis of hearing impairment[1]. Concomitantly with early diagnosis and intervention, it is important to search for the primary cause. In developed countries about 50% of the causes of isolated deafness have a genetic origin. In Brazil most of cases are due to environmental factors, such as congenital infections, perinatal asphyxia, kernicterus and meningitis[2].
Objective: To investigate the causes of hearing loss diagnosed at NHSP.
Method: Retrospective study of records of 21 deaf diagnosed from August 2003 to July 2014. Results of laboratory tests, imaging and genetic testing were analyzed.
Results: Twenty four children had a sensorineural hearing impairment, 20 were bilateral. Eight cases of newborns remained in Newborn Intensive Unit. One case showed craniofacial anomalies and 2 patients had inner ear malformations. In 3 newborn the hearing loss was due to perinatal asphyxia. Two newborns had history of infectious disease. Considering the genetic factors, 4 individuals had a history family of deafness; 3 newborns were from consanguineous family, 4 newborns were homozygous for 35delG in the *GJB2* gene, and 2 presented the A827G mutation in the *MTRNR1* gene. Three cases the etiology remains unknown.
Conclusion: The early etiological research hearing impairment contributes to support and assist disabled hearing and their families and also for assistance in public health measures.

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PM02.26

Prevalence of Non Syndromic Hearing Loss genes in a cohort of French patients

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Hearing loss is the most common sensory disorders and can affect up to 1/500 newborns. Before NGS platform could be implemented in diagnostic labs, the molecular diagnosis of non syndromic hearing loss (NSHL) was relying solely on the screening for mutations at the DFNB1 locus. Recently, we have developed a panel consisting of 71 genes known to be involved in ARNSHL, ADNSHL and X-linked NSHL. The libraries were generated with Illumina Nextera Rapid Capture Custom Enrichment or Nimblegen SeqCap EZ Choice and run on an Illumina MiSeq platform.
All NSHL patients, referred over the last 10 years and negative for DFNB1 mutations (more than 500) are currently being tested. The strategy applied is as follow: when recessive inheritance is suspected only the patient is tested by NGS; when dominant inheritance is suspected, a minimum of three relatives (the patient, affected and control family members) are tested. In all cases, validation and segregation analyses of the potential pathogenic variants is performed by Sanger sequencing.
Although X-linked HL is considered as rare, we've already identified mutations in the 3 known genes (POU3F4, SMPX and PRPS1) in 3 different families. In addition, mutations in the myosins genes are recurrent, whereas some of the other HL genes are scarcely involved. These results herald major NSHL molecular diagnosis improvements, which will directly benefit patients and families.

PS02.27

Genetically related hearing loss- results of exome sequencing in Polish patients.

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Hearing loss is an extremely heterogenic trait, approximately 50-60% of cases of hearing loss are due to genetic factors. The genes responsible for the occurrence of hearing loss usually encode proteins located in the inner ear. Majority of patients with autosomal, recessive deafness harbour mutations in only one gene- GJB2. Mutations in other genes, which products are directly involved in the hearing process, may also result in hearing loss. To date, approximately 300 genes involved in the processing of auditory information has been described, and still the new one are discovered. Efficient search for variants in the gene structure requires the use of the most modern techniques of molecular biology, such as the next-generation sequencing. Here we present the results of whole exome sequencing (WES) performed on 15 individuals suffered from familiar, prelingual hearing loss. All exome sequencing was performed on HiSeq 1500 using Illumina exome enrichment kits.

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PM02.28

Identification of novel genes and mutations associated with hearing loss in the Middle Eastern Arab population by next generation sequencing

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Introduction: One in one thousand newborns suffer from congenital hearing loss, making it the most prevalent sensory disorder in humans. To date, hundreds of mutations in more than 80 genes are associated with a hearing disability. Next Generation Sequencing (NGS) has improved the diagnostic

yield dramatically. The Middle Eastern population, comprised of different ethnic groups and known to exhibit high rates of consanguinity, has benefited from NGS. Furthermore, as many mutations first identified in this population have been subsequently found worldwide, this population is instrumental in determining genes crucial for hearing.
Materials and Methods: We applied targeted genomic enrichment along with massively parallel sequencing, encompassing 284 genes that included 121 human genes and 163 human orthologues of mouse deafness genes, on 91 families of Arab descent. One family underwent whole exon sequencing (WES).
Results: This research uncovered novel mutations in 23 known deafness genes. A novel gene, *SLC25A21*, associated with hearing loss was detected by WES. We were able to determine the causative mutation for deafness in 32% of our population.
Conclusions: NGS is an optimal technology for genetic diagnosis of hearing loss. Challenges remain in the search for mutations responsible for deafness: to identify the causative variant, particularly when several variants exist and segregate in the family; to detect structural variations and regulatory elements and to prove their involvement in hearing impairment; and to prove mutations in a novel gene causes hearing loss by relevant functional assays.
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PS02.29
The contribution of GJB2 gene mutations to development of early onset hearing loss in affected group of patients in Lithuanian population.

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Congenital hearing loss (CHL) is one of the most common traits diagnosed 1/1000 newborns. Genetic factors contribute to 2/3 of CHL cases in industrialized countries. Mutations of GJB2 gene are a major cause of CHL worldwide.
The aim of the study is to establish the contribution of GJB2 gene mutations to development of early onset HL in affected group of patients in Lithuanian population.
Objectives: to determine the incidence and structure of GJB2 mutations in patients affected with early onset nonsyndromic HL, to analyze audiologic features, genealogies and perform genotype - phenotype analysis.
Methods: Clinical data and GJB2 gene sequencing results were obtained from a retrospective collection of 133 patients (122 unrelated probands) with early onset nonsyndromic HL compiled in VUH Santariškiu clinics 2010-2014. Statistic summary, homogeneity tests, and logistic regression analysis were employed for the assessment of genotype-phenotype correlation.
Results: Our findings show high proportion of GJB2-positive patients (60.1%) in the study group. Most prevalent GJB2 mutations were c.35delG and c.313_326del14 (67.6% and 25.8% of mutated alleles). The statistical analysis revealed significant differences between GJB2-positive and GJB2-negative groups in disease severity (p=0.02), symmetry (p=0.04), and family history (p=0.0035). The probability of identifying GJB2 mutations in patients affected with profound/severe vs moderate/mild HL and positive vs negative family history estimated to be 2.773(95%CI 1.26-6.192;p=0.0116) and 3.235(95%CI 1.478-7.436;p=0.0418) respectively.
Conclusion: The findings of the study quantified the impact of GJB2 mutations to the development of HL and are useful for setting up the principles for the prediction of disease course in Lithuanian population.

PM02.30
NGS revealed PSIP1/LEDGF as a new gene causing sensorineural progressive hearing loss and variable eye phenotypes

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Hereditary Hearing Loss (HHL) is an extremely genetically heterogeneous disorder that prompted us to develop a powerful diagnostic algorithm characterized by screening of 113 HHL genes by targeted re-sequencing followed, in negative cases, by whole exome sequencing to detect causative mutation in new genes. Thanks to this strategy we identified PSIP1/LEDGF as a novel gene causing sensorineural progressive HHL restricted to the medium-high frequencies and a variable eye phenotype (i.e. uveitis, optic neuropathy) in an ltalian pedigree. Further clinical examinations of the affected members showed normal amplitude of the ABR indicating no involvement of the auditory nerve while VEP indicated some differences in terms of visual acuity and optic nerve functionality.
A frameshift deletion leading to a premature stop codon (c.1554_1555del, p.E518Dfs*2, p.T519X) with truncation of the last 12 amino acids and segregating with the disease was detected. Our additional studies using different methodological approaches (i.e. cDNA analysis, RNA Seq, immunolabeling, etc.) demonstrate that: 1) this deletion does not lead to mRNA degradation 2) Psip1 is expressed in the nuclei of all hair cells and supporting cells of both mouse cochlea and vestibular system with the exclusion of the auditory nerve.
Recently, PSIP1 was described as a transcriptional co-activator regulated by miR-135b in cells of the mouse inner ear and as a possible protector against photoreceptor degeneration. Present findings strongly suggest an important role of PSIP1 in HHL as well as in eye defect and further highlight the reliability of our strategy to study the genetic basis of HHL

PS02.31
A Next Generation Sequencing amplicon-based strategy to explore Inherited Retinal Degeneration complexity

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Inherited Retinal Degeneration (IRD) are a group of eye disorders, characterized by photoreceptors degeneration which include: Retinitis Pigmentosa, Stargardt disease, Usher Syndrome and Leber congenital amaurosis. The high genetic heterogeneity, the incompleteness of disease specific databases and the elevated number of genes involved in IRD, often hamper the correct molecular diagnosis and patients stratification.
To clarify IRD molecular profile, we used a next Generation Sequencing (NGS) strategy and designed a custom AmpliSeq panel (Life Technologies), containing the coding sequences of 72 disease related genes, for a total of 1649 amplicons.
An in-house bioinformatic pipeline was optimized to filter synonymous variants and polymorphism and to annotate variants with prediction algorithm (dbNSFP) and disease specific databases (LOVD eye diseases, Retina International, RPGR database).
A cohort of 40 samples was selected (29 patients, 11 healthy relatives). They underwent a complete ophthalmologic examination (visual acuity, anterior segment and fundus examination, ERG and/or EOG, OCT), as well as a genetic counselling.
Possibly causative mutations were detected in 62% patients (n=18). We found mutations in 8 genes. The most recurrent gene was mutated in 38% (n=7) of patients. The remaining seven genes harboured lower frequencies with just one or two patients mutated. Overall, seven genes were inherited with an autosomal recessive pattern and one gene was X-linked.
Of note, less than 21% of variants have been already described in specific databases. These preliminary results highlight the need to further explore the molecular complexity and heterogeneity of RD in order to translate these analyses into clinical practice.
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PM02.32
Evidence against *ZNF469* being causative for keratoconus

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ZNF469 has been associated with Brittle cornea syndrome and central corneal thickness. The latest study also revealed that rare heterozygous mutations in *ZNF469* determine keratoconus (KTCN) susceptibility etiology. KTCN is a degenerative disorder of the eye characterized by stromal thinning and protrusion of the cornea, resulting in severe impairment of visual function. To investigate the contribution of *ZNF469* to KTCN, we Sanger sequenced *ZNF469* in 42 Polish KTCN patients and 49 Polish individuals with high myopia (HM) as a control group. The average number of *ZNF469* variants per individual was 40.59520 and 40.26531 for KTCN and HM, respectively (Wilcoxon Rank Sum test, p = 0.3601). Moreover, the average number of nonsynonymous variants was 16.30952 and 16.0 for KTCN and HM, respectively (Wilcoxon Rank Sum test, p = 0.3724). All identified variants were previously reported. Minor allele frequency (MAF) was determined based on the whole exome sequencing results from 268 Polish patients without ocular abnormalities. Among missense variants, only one (rs528085780) has MAF ≤ 0.01 and has been identified in one patient with sporadic KTCN. However, the resulting Arg1864Lys substitution was not predicted to be deleterious based on different prediction algorithms and conservation scores. Summarizing, we have not found a significant enrichment of pathogenic variants in *ZNF469* in KTCN patients. High prevalence of *ZNF469* variants identified in our KTCN group is typical for a common genetic variation observed in general population. Genetic factors different than variation in *ZNF469* are responsible for KTCN development.

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PS02.33

Multiplex detection of mitochondrial variants by MALDI-TOF MS in Brazilian patients with hereditary optic neuropathy

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Leber's hereditary optic neuropathy (LHON) is a mitochondrial disease characterized by bilateral loss of vision. Over 95% of LHON cases are associated with one of three main mitochondrial DNA (mtDNA) mutations, G11778A, T14484C, and G3460A. Other mutations appear in 5% of cases. The objectives of this study were: to determine the frequency of LHON mutations, to identify mitochondrial haplogroups in Brazilian patients and to evaluate the usefulness of iPLEX Gold/MALDI-TOF MS technology in detecting LHON mutations. We analyzed a total of 101 patients, 67 with LHON clinical diagnosis and 34 with optic neuropathy of unknown etiology. The techniques used were PCR-RFLP, direct sequencing and iPLEX Gold/MALDI-TOF MS. The frequency of the LHON mutations observed was 83% for G11778A and 17% for T14484C. Our findings contributed to confirm the LHON clinical diagnosis of 36 patients (36%) and allowed to clarify the diagnosis of 12 patients (35%) with optic neuropathy of unknown etiology. The most frequent haplogroups were of African origin: L1/L2 and L3. The frequency of the LHON mutations and haplogroups found in the study are relatively different from other published data in other parts of the world. Furthermore, the iPLEX Gold/ MALDI-TOF MS technology platform proved to be a very accurate and efficient method to screening of the mutations and identification of the haplogroups related to LHON. The platform combined with other techniques was important to elucidate approximately 35% of the cases studied.

PM02.34

Two cases of duplication 6p25.3, expanding the phenotype of FOXC1 duplication syndrome

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Loss of function due to mutations in the *FOXC1* gene are known causes of dysgenesis of the anterior segment of the eye. Very few is currently known about duplications involving the whole *FOXC1* gene. Only two families have been previously described in which FOXC1 duplication is associated with iris hypoplasia (MIM 308500). No further phenotypic features being reported (*Nishimura, 2001*). This Copy Number Variant (CNV) is very rarely reported in the major databases as only two cases are noted in Decipher with only one phenotype available.

We report two additional unrelated cases with complete *FOXC1* duplication found by array CGH. The first patient displays a 4Mb duplication of the region 6p25.3, and a 7Mb deletion in the region 12p13.33p13.31. She presented with dysgenesis of the anterior segment of the eye and a keratoconus, intrauterine and post natal growth retardation, atrial septal defect, developmental delay, dysmorphic features and inner ear malformation.

The second patient displays an isolated duplication of the region 6p25.2p25.3 spanning 1,195 Mb. She had a congenital strabismus, unilateral ptosis with amblyopia, bilateral neurosensory deafness, developmental delay, behavioral disturbances, and dysmorphic features.

Both patients displayed overlapping phenotypic features with the ones previously described.

Further investigations are needed to determine the frequency of such characteristics and their consistency in *FOXC1* duplication syndrome. This duplication appears to be linked to developmental abnormalities as well as a loss of function mutations. This suggests that the normal development of at least the eye requires no more than two copies of the *FOXC1gene*.

PS02.35

Identification of a new case of colobomatous microphthalmia with recessive null mutations in TNEM3 by targeted gene analysis

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Microphthalmia and anophthalmia are the most severe malformations of the eye. Analysis of the genes currently associated to microphthalmia or anophthalmia leads to the identification of a pathogenic mutation in only 25% of cases. By combination of autozygome and exome analysis, a homozygous loss of function mutation in the TNEM3 gene (previously named ODZ3) was reported in two sibs with isolated bilateral colobomatous microphthalmia from a consanguineous Saudi family (Aldahmesh MA et al., Genet Med 2012). ODZ3 is strongly expressed in the optic stalk in vertebrates. Mice partially knocked down for the gene ODZ3 (lacking only the transmembrane domain) have morphologically normal eyes but impaired binocular vision. A new generation sequencing approach targeted on a panel of 180 genes definitely or possibly implicated in microphthalmia or anophthalmia was performed on a cohort of 96 patients, and identified a third patient harboring a TENM3 homozygous null mutation (c.2968-2A>T). This patient had bilateral colobomatous microphthalmia, with anterior chamber malformation and chorioretinal coloboma, associated with mild intellectual disability. The identified mutation (c.2968-2A>T) affects a consensus acceptor splice site. This result supports the role of TNEM3 in human microphthalmia. The three patients described to date with homozygous TENM3 loss of function mutations present with a similar phenotype of bilateral colobomatous microphthalmia.

PM02.36

Whole exome sequence analysis of 106 families with microphthalmia, anophthalmia and/or coloboma (MAC)

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Whole exome sequencing (WES) was performed on 106 MAC families that comprised two extended pedigrees, 22 trios, 12 affected-relative pairs, and 70 singletons. Samples were pre-screened for mutations in *SOX2*, *OTX2* and *PAX6* (paired domain): the major genes in severe bilateral MAC.

Sample preparation was performed in-house or at the Wellcome Trust Sanger Institute as part of the UK10K project, which generated 75 of the exomes. Sequence alignment/variant filtering were performed as previously described (PMID24462371). Targeted resequencing of more than 400 unrelated MAC cases was performed to identify additional/recurrent alleles of the exome-indicated genes.

Pathogenically significant variants are identified and validated in 22 genes to date, including the confirmed eye genes listed. Analyses to identify *de novo* mutations indicate genes newly associated with developmental eye defects.

Gene	Pathogenic genotypes: exome	Pathogenic genotypes: resequencing
FOXE3	1 AR singleton	-
STRA6	1 AR singleton	-
ALDH1A3	4 AR singletons	3 cases
RARB	1 AD singleton	4 AD cases
MAB21L2	1 affected-relative pair	3 cases
YAP1	2 cases	-
ADAMTS18	1 AR singleton	-
FZD5	1 extended pedigree	-
BMP7	2 cases	-
ACTG1	2 cases	-
GJA8	1 singleton	3 cases
SOX2	1 singleton (CNV)	-

The cohort size has facilitated the identification of variants in genes with a known or novel association with MAC. Mutations in confirmed eye genes account for 17% of WES MAC cases.

PS02.37
Isolated microphthalmia with coloboma of optic nerve mapped to a locus on chromosome 2.

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Large kindred of the Jewish Iranian inbred Mashhad community presented with an apparently autosomal recessive phenotype of severe microphthalmia and coloboma of optic nerve. Homozygosity mapping using Affymetrix 250K SNP arrays identified a single ~2.5Mb disease-associated locus on chromosome 2 between rs4672834 and rs517656411. Multipoint LOD score was calculated and yielded a score of 3.2 at D2S433. Interestingly, ABCB6 (MIM# 614497), encoding an ATP-binding cassette and known to be associated with autosomal dominant microphthalmia, lies within the defined locus. However, Sanger sequencing of ABCB6 and its promotor revealed no mutations. Whole exome sequencing was performed and data analysis is being processed.

PS02.39
Interplay between SOX10 and p54NRB:molecular and cellular consequences in the context of Waardenburg syndrome and related disorders

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SOX10 is a transcription factor (TF) with a crucial role in Neural Crest (NC) development. Mutations within this member of the SOX family were first associated with Waardenburg-Hirschsprung disease (WS4, deafness, pigmentation defects and intestinal aganglionosis). Variable phenotypes that extend beyond the initial limits of this syndrome and incomplete penetrance of each feature are now reported. In addition to WS4 or WS2 (Waardenburg syndrome without Hirschsprung disease), some patients present with central and/or peripheral myelination defects. While an escape from the NMD pathway was proposed to explain the severe phenotypes in case of truncating mutations, no mechanism has been anticipated for missense mutations so far. In vitro studies confirmed deleterious effects of the latter on the main SOX10 functions, while about half of the resulting proteins are redistributed in nuclear bodies (foci) of undetermined nature and function.
Here, we report that p54NRB (NONO) interacts with SOX10 and acts in synergy with it to activate the expression of several target genes during NC development. Interestingly, p54NRB co-localizes with all foci forming SOX10 mutants . However, siRNA experiments indicated that p54NRB is not essential for their formation. Two other members of the DBHS protein family also co-localise with SOX10 mutants, raising the possible paraspeckles nature of the foci or the re-localisation of the DBHS members in other sub-nuclear compartments. Of note, WT SOX10 protein is also sequestrated in foci, leading to altered synergistic activity between this TF and p54NRB. We speculate that the dominant negative effect we observe could contribute or be at the origin of the neurological phenotypes observed in patients.

PM02.40
Next-generation sequencing of the ABCA4 gene reveals a high frequency of complex alleles and novel mutations in Polish patients with retinal dystrophies

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Variation in the ABCA4 locus has emerged as the most prevalent cause of monogenic retinal diseases. Using next-generation sequencing targeting ABCA4, the first such approach in Polish patients with Stargardt disease (STGD, n=76) and cone-rod dystrophy (CRD, n=16), together with a Polish population exome data (n=594), we disprove the pathogenic status of p.V552I and provide more evidence against a causal role of six further ABCA4 variants as drivers of the phenotype under a recessive paradigm. Our study identifies 12 novel potentially pathogenic mutations (four of them recurrent) and a novel complex allele p.[(R152*; V2050L)]. In one third (31/92) of our cohort we detected the p.[(L541P; A1038V)] complex allele, representing an unusually high level of genetic homogeneity for ABCA4-related diseases. A combination of p.[(L541P; A1038V)] and/or a truncating change always resulted in an early disease onset. The comprehensive, population-specific study expands our knowledge on the genetic landscape of retinal diseases.
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PS02.41
Identification of four novel NHS protein-truncating mutations in families with X-linked cataract (CXN) and Nance-Horan syndrome (NHS) reflects the variable expressivity of the disease

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Nance-Horan Syndrome (NHS) and X-linked cataract (CXN) have previously been reported to be allelic disorders, caused by NHS protein truncation and aberrant *NHS* transcription, respectively. In this study, five families diagnosed with NHS and one CXN family were recruited. All coding exons and splice sites of the *NHS* gene were PCR amplified and Sanger sequenced. Whole exome sequencing (WES) was performed for *NHS*-mutation negative patients. The WES dataset was filtered for rare variants (minor allele frequency ≤0.01) in congenital cataract genes, with reference to Cat-Map and GenomeTrax. Direct sequencing of *NHS* coding exons revealed mutations in 4 families; two novel frameshift mutations, p.(Val1187fs*1187) and p.(Leu256fs*284), and a segmental deletion of Xp22.13 encompassing *NHS*. Although the segmental deletion also disrupted four neighbouring genes *SCML1*, *SCML2*, *BEND2* and *RAI2*, this family did not present a clinically distinct phenotype, compared to families with disruption of *NHS* alone. Interestingly, a nonsense mutation, p.(Q175*), was identified in a CXN family lacking any of the extra-ocular phenotypes observed in NHS patients. WES analysis of *NHS*-negative patients did not reveal any variants in a common candidate gene. Further investigation of the WES data is required, although a mutation in a non-coding region or copy number variation of the *NHS* gene cannot be fully excluded. This study suggests the possible genetic heterogeneity of an NHS-like cataract-dental syndrome. Variable disease expression observed in the CXN family with an *NHS* stop mutation demonstrates that the phenotype-genotype correlation may not be as constricted as originally suggested.

PM02.42
Towards the genetic homogeneity of neonatal severe pseudocoloboma of the central retina with early-onset optic atrophy (LCA9).

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Introduction: NMNAT1 encodes a homohexameric NAD- synthesizing enzyme as well as a chaperone that protects against neuronal activity-induced degeneration. NMNAT1 mutations cause a highly specific Leber congenital amaurosis phenotype characterized by severe neonatal neurodegeneration of the central retina with early-onset optic atrophy. The purpose of this study was to search for copy number variations (CNV) and mutations affecting 5' regulatory elements or splicing of the gene in 22 single heterozygote index cases with the NMNAT1 phenotype.
Results: Three unique CNVs, i.e. a 1-exon duplication, a 1-exon deletion and a two exon-deletion were identified in 3/22 patients. In addition, 5 distinct variants lying in the 5'UTR of the gene were identified in 10/22 individuals. 1/5 UTR variant was shared by 6/10 individuals originating from the French Indian Ocean Island, La Réunion. This variant was undetectable in all available patient cDNAs (3/6), supporting down regulation or instability of the mutant mRNA. mRNA from 2/10 other individuals harboring other UTR variants were available which analysis was consistent with similar mRNA defects.
Conclusion: Here, we report that at least 13/22 LCA individuals with single heterozygote NMNAT1 mutations carried a second disease allele undetected by Sanger sequencing of the NMNAT1 exome. This result suggests that severe neonatal neurodegeneration of the central retina with early-onset optic atrophy is pathognomonic of NMNAT1 mutations. In-depth molecular ana-

lysis of the gene and surrounding regulatory elements should be considered in all patients harboring this highly specific LCA phenotype.

PS02.43
Cone structure and function investigation in family with an RP1L1 mutation

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Screening by next generation sequencing for retinal dystrophies in a 23 year-old female with intense photophobia and progressive bilateral visual loss, identified a RP1L1 gene mutation: p.Arg45Trp. The same genetic variant was found in three family members. To examine cone structure and function in all family members, we obtained high-resolution retinal images of the cone mosaic with a flood-illumination adaptive optics (AO) retinal camera, over 2 years. Best corrected visual acuity (BCVA), contrast sensitivity function (CSF), combined SLO and SD-OCT imaging, MP1 microperimetry and multifocal electroretinography (mfERG) were also performed.
The complementary use of objective tools for analysis of *en face* and *cross-section* high-resolution views of the photoreceptor layer confirmed an asymptomatic phenotype in two family members and detected a mild phenotype in the proband's father. Our findings led to diagnosis of occult macular dystrophy (OMD) in this family.
RP1L1 gene mutations associated with OMD and the p.Arg45Trp variant have been reported in ethnically diverse families with different occult macular dystrophy segregation patterns. Using new high-resolution adaptive optical imaging, we detected a heterogeneous phenotype with different central cone morpho-functional abnormalities across family members harboring the p.Arg45Trp variant. The different phenotypes in this family may be ascribed to incomplete penetrance of the RP1L1 gene variant. Although we found only this variant analysing 61 genes, we cannot exclude that mutations in other genes might contribute to the phenotype.

PM02.44
Functional characterization of two novel splicing mutations in the SLC45A2 gene associated with Oculocutaneous Albinism Type IV

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Oculocutaneous albinism (OCA) is characterized by hypopigmentation of the skin, hair and eye, and by ophthalmologic abnormalities caused by a deficiency in melanin biosynthesis. OCA is an autosomal recessive inherited condition and four principal forms (OCA1-4) are recognized based on the presence of mutations in *TYR*, *OCA2*, *TYRP1* and *SLC45A2* genes respectively.
In this study, we investigate the molecular basis of OCA4 in one Italian albino patient. Sequencing analysis of the *SLC45A2* gene identified two hitherto-unknown putative splicing mutations. The first one lies in the consensus sequence of the donor splice site of *SLC45A2* intron 5 in compound heterozygosity with a synonymous transition involving the last nucleotide of exon 3. *In-silico* prediction of the effect of both mutations on splicing shows a score reduction for the mutant splice sites and indicate the possible activation of newly-created splice sites. The effect on splicing of these two novel mutations is going to be investigate using an *in-vitro* hybrid-minigene approach to lead to the demonstration of their causative role and to the identification of aberrant transcript variants. Although the in-vitro characterization of splicing mutations may not completely represent the splicing events that really occur in vivo, it certainly provides a strong support to the pathogenic role of the identified variants.

PS02.45
OPA1 testing in optic atrophy: a 5-year service summary

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OPA1, a nuclear gene encoding a mitochondrial dynamin-related GTPase, is the major cause of autosomal dominant optic neuropathy, manifesting in a spectrum ranging from isolated optic atrophy to complex multi-system neurological disease. We have been providing comprehensive clinical testing for *OPA1* in the UK and internationally since 2009, using a combination of amplicon-based massively parallel sequencing, Sanger sequencing, and MLPA dosage analysis. Full screens have been performed on 290 apparently unrelated consecutive cases/families, with requests originating from Clinical Genetics, Ophthalmology, Neurology, and specialist tertiary centres. Pathogenic or likely pathogenic outcomes were seen in 66 cases/families - a working detection rate of 23%, which may indicate more relaxed clinical filtering and/or increased affordability of testing recently, nevertheless remaining relatively high. Local testing for familial mutations was carried out in 40 individuals. All 84 sequence and structural changes encountered, including 42 novel ones, have been recorded and evaluated systematically, comprising a curated set for data filtering and scoring. Apart from established polymorphisms, they have also been deposited in relevant clinical databases, adding to the publically available mutation/variation spectrum of *OPA1*.

PM02.46
The hearing phenotype associated with an in-frame deletion in FOXL1 causing autosomal dominant otosclerosis

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Introduction: Clinical otosclerosis, a bone remodeling disease of the human temporal bone, involves hearing loss, usually with a conductive impairment associated with fixation of the stapes. Other regions of the otic capsule may also be involved, with variable clinical presentation and progression.
Materials & Method: Subjects were a multiplex AD family from the island of Newfoundland identified with a 15 bp in-frame deletion in the FOXL1 gene, and an Ontario proband identified with the same mutation from a cohort of 32 Ontario cases with surgically confirmed otosclerosis. Phenotype data included retrospective data acquisition from clinical charts (Newfoundland and Ontario) and prospective measures (Ontario) including behavioural audiometry, middle ear analyses (immitance and middle ear muscle acoustic reflexes) and otoacoustic emissions.
Results: Phenotype analyses revealed a bilateral hearing loss in the Newfoundland proband, initially conductive in nature and later progressing to a mixed hearing loss. Newfoundland relatives with surgically confirmed otosclerosis (n=6) also exhibited a bilateral impairment, primarily of the conductive or mixed types; 2 cases did not undergo surgery and were considered non-penetrant. The Ontario proband presented with a profound mixed loss in the right ear, and a mild sensorineural hearing loss in the left ear; acoustic middle ear muscle reflexes were absent despite normal middle ear compliance.
Conclusions: Subjects with the FOXL1 mutation exhibit the hallmark features of clinical otosclerosis. Physiological measures of middle ear function can be used to supplement the behavioural phenotype, and may enable early detection of otosclerotic pathology.

PS02.47
PRDM12 is an epigentic regulator and a cause of congenital insensitivity to pain

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We report a new Mendelian disorder causing painlessness and an inability to sense temperature. Presentation is with temperature instability, self mutilations and frequent accidents and corneal trauma. The condition has similarities to HSAN4 and HSAN5 with the following important differences: there is a loss of small myelinated, not small unmyelinated, nerves; itch is not a feature; and mental retardation is rare. We found bi-allelic mutations in the histone methyletransferase PRDM12. We will describe the phenotype and genotype of this new condition, and how to distinguish it from similar disorders. As we have found that PRDM12 is expressed in post-natal nociceptors it si likely to be a new analgesic target.

PM02.48
Expanding the genotypic spectrum in Perrault syndrome

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Perrault syndrome is a rare autosomal recessive disorder characterised by sensorineural hearing loss (SNHL) in both sexes and primary ovarian insufficiency in 46, XX karyotype females. Perrault syndrome is genetically heterogeneous with variants in five genes reported to date, *HSD17B4*, *HARS2*, *LARS2*, *CLPP* and *C10orf2*. Subsequent to the original discovery studies, no further cases of Perrault syndrome due to variants in these genes have been reported. Here we present four families affected by Perrault syndrome with novel and previously reported mutations in *HSD17B4, LARS2* and *C10orf2*. The proband from each family was whole exome sequenced and the mutations confirmed by Sanger sequencing. A female from family P1 was compound heterozygous for a known and novel mutation in *HSD17B4*, c.46G>A, p.(Gly16Ser) and c.244G>T, p.(Val82Phe), respectively. Two families (P2 and P3) had biallelic mutations in *LARS2*. The affected female and male in P2 were homozygous for c.1565C>A, p.(Thr522Asn), previously associated with Perrault syndrome. Family P3 consisted of an affected female and male, who were compound heterozygous for c.1565C>A, p.(Thr522Asn) and c.351G>C, p.(Met117Ile). Affected members of both families showed a low frequency SNHL an unusual audiometric configuration previously described in Perrault syndrome caused by *LARS2* mutations. A further affected female (family P4) with significant neurological disability was compound heterozygous for mutations in *C10orf2*, c.968G>A p.(Arg323Gln) and c.1196A>G p.(Asn399Ser). These cases independently confirm *HSD17B4*, *LARS2* and *C10orf2* as causative of Perrault syndrome.

PS02.49

Association of novel mutation p.W325X (c.977G>A) in the POU3F4 gene with perilymphatic Gusher-deafness syndrome (DFNX2) in Yakut family (Eastern Siberia)

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The most rare causes of deafness in the world are X-linked and mitochondrial forms of hearing impairment (1-2%). For the first time, we revealed identical abnormalities of inner ear in two brothers from Yakut family (Eastern Siberia) with presumably X-linked recessive deafness that are typical for the perilymphatic Gusher-deafness syndrome (DFNX2, MIM304400) caused by mutations in the POU3F4 gene (MIM 300039). Computed tomography studies demonstrated an abnormal dilatation of the internal acoustic canal (IAC) as well as an abnormally wide communication between the IAC and the inner ear compartment in both brothers. Parents had no any temporal bone anomalies. To determine whether mutations in the POU3F4 gene (Xq21.1) are responsible for the hearing loss in these patients, we sequenced the single exon of the POU3F4 gene. We identified novel nucleotide substitution c.977G>A in POU3F4 in hemizygous state in both probands, in the heterozygous state in their mother, and c.977G>A was absent in their father. Transition c.977G>A leads to stop codon (p.W325X) in the POU-homeodomain of human transcription factor protein. Segregation of inner ear malformations and novel mutation c.977G>A (p.W325X) in the POU3F4 gene with deafness in studied Yakut family confirmed association of the POU3F4 gene with clinical phenotype of perilymphatic Gusher-deafness syndrome (DFNX2, OMIM 304400). Study was supported by RFBR (#14-04-01741_A, 14-04-9010_Bel_A, 15-44-05106-r_vostok_a), Governmental contract # 6.656.2014/K, SBRAS Integration project #92 and Grant of the Head of the Republic of Sakha (Yakutia) for young scientists, experts and students for 2015 years (RG#76 from 02.06.2015).

PM02.50

Genome-wide association study and targeted re-sequencing: a new combined approach to investigate the genetic causes of Age-related Hearing Loss (ARHL)

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ARHL is a degenerative disease affecting millions of people worldwide. To date, very few potentially causative genes have been described. Based on our previous GWAS and expression data, a custom targeted re-sequencing panel of 46 ARHL candidate genes was designed and used for screening 500 ARHL Italian patients coming from both inbred and outbred populations using Ion Torrent PGM™ technology. Data were filtered according to allele frequency and to pathogenicity prediction (i.e. in silico predictor tools). Twenty-one mutations located in 14 different genes (8 frameshift, 2 stop gain and 11 missense) affecting 49 patients were detected. All mutations were absent in controls coming from the same populations as well as in any public database (or present at a very low frequency MAF≤0,003). Missense mutations were predicted to be highly damaging. Both scenarios were present: different mutations in the same gene as well as the same mutation in different patients. In some cases, human phenotype resembles that of already existing mice models. For example, a) 3 novel heterozygous frameshift deletions were detected in XIRP2 in patients showing a very similar phenotype to that recently described in mice (Francis et al. 2015); b) a heterozygous missense mutation was detected in four patients showing a high-frequency HL in WBP2 gene, similar to that described in the corresponding mouse model (Buniello et al. 2014). Complete results will be presented. These findings demonstrate the usefulness of our approach (GWAS+ Expression studies + Mutation screening) further supporting the potential role of these genes in causing ARHL.

PS02.51

New candidate genes associated with primary congenital glaucoma

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Primary congenital glaucoma (PCG) is an ocular inherited autosomal recessive disease, associated with CYP1B1 mutations. However, some of the patients do not have CYP1B1 mutations. This study aims to identify novel genes causative of PCG in these patients.

Whole exome sequencing was performed in three unrelated patients and one trio. After annotation and variant filtration according to the autosomal recessive disease model, rare variants with a pathogenic functional impact were selected.

The comparison of variants shared by the four patients did not identify a common mutated gene. The four samples had 4, 9, 5 and 8 altered genes, respectively. None of these genes were previously associated with glaucoma, except an RNASEH2C gene variant, mutated in one patient and confirmed in the trio analysis. This gene has been associated with Aicardi-Goutières syndrome, characterized by severe neurological abnormalities together with congenital glaucoma. However, the patient does not have a typical phenotype. According to in silico previsions, this mutation leads to the loss of a donor splice site, creating a longer protein. Studies are underway to characterize the RNASEH2C mutation in this family.

Since no common mutated gene was found in our study and other reported studies, we suggest that PCG may be caused by mutations in different genes, consistent with an autosomal recessive model, or by an alternative model such as the polygenic, with heterozygous variants in different genes.

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PM02.52

Heterozygous RARB missense mutations causing microphthalmia: Four further cases to add to this emerging phenotype

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Background

RARB (Retinoic Acid Receptor B) plays an important role in eye development. Compound heterozygous loss of function mutations in RARB have been associated with PDAC syndrome (pulmonary hypoplasia/agenesis, diaphragmatic hernia/eventration, anophthalmia/microphthalmia, and cardiac defect). Three unrelated subjects with microphthalmia and dia-

phragmatic hernia have been described with de novo, gain of function missense mutations affecting the same codon of RARB (Arg387). Two of these patients (both with p.Arg387Cys substitutions) died in utero and after a few hours of life respectively. The third patient (p.Arg387Ser) was 16 years old when his case was described in the literature.
New cases
We describe four new cases of missense RARB mutations at Arg387 in patients with microphthalmia/anophthalmia. One patient is an adult male of normal intelligence and a p.Arg387Ser substitution. He does not have a known diaphragmatic hernia. The other three patients all have p.Arg387Cys substitutions.
These four cases add to an emerging phenotype associated with RARB missense mutations.

PS02.53

Recessive RHO mutation E150K and SAMD7 regulatory variants in a consanguineous family with retinitis pigmentosa

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Purpose
To identify the genetic cause of a retintis pigmentosa phenotype observed in a Turkish consanguineous family.
Methods
Homozygosity mapping was performed for five sibs, two of which are affected. Functional analysis of non-coding SAMD7 variants was performed by luciferase assays and electroporation assays in mouse retinal explants with SAMD7 CBR-reporter constructs (Hlawatsch et al. 2013).
Results
Homozygosity mapping revealed two candidate genes, namely RHO and SAMD7. A homozygous RHO mutation (c.448G>A, p.E150K) was found in the two affected sibs, while all other sibs were heterozygous carriers.
No coding SAMD7 mutations were found. Interestingly, sequencing of the SAMD7 promoter and an enhancer in the two affected sibs revealed four homozygous variants located in the binding regions of the CRX transcription factor. The variants are known SNPs, with a low MAF of 1,6 %. The first three SNPs are located in a CRX-binding region called CBR1, while the fourth SNP is located in CBR2. The combined SAMD7 CBR1/CBR2 mutated construct showed a significantly decreased SAMD7 reporter activity compared with the WT construct.
Conclusions
A rare recessive RHO mutation (E150K) was found in consanguineous RP patients, consistent with previous reports (Kumaramanickavel et al., 1994; Azam et al., 2009 and Zhang et al., 2013). Moreover, functional analysis of four variants located in non-coding, CRX-binding regions of SAMD7, suggested a regulatory and synergistic effect on SAMD7 expression. As Samd7 has recently been identified as a novel Crx-regulated transcriptional repressor in retina (Hlawatsch et al., 2013), we hypothesize that these SAMD7 variants might have a modifying effect on the retinal phenotype observed in this family.

PS02.55

Molecular diagnosis of complex Eye disorders by Next Generation Sequencing

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Molecular detection of diseases such as eye disorders is very challenging. Hunting the right gene in these complex disorders is like finding the needle in the haystack. Nevertheless, modern sequencing platforms known under collective name Next generation sequencing or NGS is a huge help to solve this problem. We also could be able identifying disease causing mutations by NGS in different and unrelated retinopathy cases. In One case, the PRPH2 gene was homozygous mutated at nucleotide c.582-1(G>A) in 2 affected children of related parents. This change is a splice site variation that leads to vitelliform macular dystrophy. Further, we found in other family in the CRB1 gene mutation at c.T2106G (p.Y702X) causing macular dystrophy. Third case showed pathogenic mutation in the CNGA3 gene at c.A827G (p.N276S) causing achromatopsia type 2. A affected male was mutated in the gene TULP1 at position c.1450A>C (T484P) causing Leber Congenital Amaurosis (LCA) or RP type 14 and further affected female was positive for a change in the RDH12 gene at c.81delT (p.A27fs). Mutations in this gene cause Leber Congenital Amaurosis. All mentioned mutations are unreported. To validate pathogenic nature of these changes, we analyzed the mutations by Sanger sequencing and some predicting software. In addition, missense mutations were compared for protein structure changes with mutated and wild type amino acid. In all cases we observed significant difference in protein folding, which might influences the biological function. Furthermore, over 100 normal population samples were tested with negative results for each case.

PM02.56

Two missense mutations in SALL4 in a patient with microphthalmia, coloboma and optic nerve hypoplasia

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There are four human homologues of the D. Melanogaster Sal gene that are highly conserved, C2H2 double zinc finger transcription factors. Mutations in SALL1 causes Townes-Brocks syndrome, SALL2 and SALL3 have not been associated with human disease and mutations in SALL4 cause a phenotypic spectrum that includes Okihiro/Duane-radial ray syndrome, acro-renal-ocular syndrome and Holt-Oram syndrome. As part of our studies into the etiology of anophthalmia, microphthalmia and coloboma (AMC), we used exome sequencing with a trio approach in a Caucasian female with unilateral microphthalmia and coloboma, bilateral optic nerve hypoplasia, a ventricular septal defect, atrial septal defect and growth delays. Prior investigations included normal array CGH and both parents reportedly had no clinical features. We found two sequence variants in SALL4 - c.575C>A, predicting p.Ala192Glu and c.G2053G>C, predicting p.Asp685His (NM_020436). Both variants were verified using Sanger sequencing and predicted to be damaging with three software programs; the first was paternally inherited and the second was maternally inherited. Only p.Ala192Glu was present in normal controls (6/121,266). Structural eye defects have been infrequent in individuals with SALL4 mutations. In two families diagnosed with acro-renal-ocular syndrome, segmental disc dysplasia and hypoplasia and retinal coloboma were found with a SALL4 deletion and cataract, iris and choroidal colobomas, microphthalmia and microcornea were reported with heterozygosity for a frameshift mutation. As Sall4 regulates Bmp4, we speculate that altered BMP4 expression could be responsible for the eye defects and conclude that haploinsufficiency for SALL4 should be considered as a rare cause of AMC.

PS02.57

An augmented ABCA4 screen targeting non-coding regions reveals a deep intronic founder causal variant in Belgian Stargardt patients

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Autosomal recessive Stargardt disease (STGD1) is hallmarked by a large proportion of patients with a single heterozygous causative variant in the disease gene ABCA4. Braun et al. (2013) reported deep intronic variants of ABCA4, prompting us to perform an augmented screen in 131 Belgian STGD1 patients with one or no ABCA4 variant to uncover deep intronic causal ABCA4 variants.
All 131 prescreened patients underwent targeted resequencing of four deep

intronic ABCA4 regions using next-generation sequencing (Miseq, Illumina), revealing a second variant in 28.6% of cases. Twenty-six percent of these carry the same causal variant c.4539+2001G>A (known as V4). Haplotyping in V4 carriers showed a common region of 63 kb, suggestive of a founder mutation. Genotype-phenotype correlations indicate a moderate-to-severe impact of V4 on the STGD1 phenotype.
The remaining patients in whom no second ABCA4 mutation was identified underwent targeted resequencing of the entire genomic regions of ABCA4, BEST1 and PRPH2 (~ 500 kb in total) (HaloPlex Target Enrichment System, Agilent Technologies). Variant filtering was based on minor allele frequency, conservation score, splicing predictions and regulatory potential. Preliminary data in a pilot group show that the standard filtering yields approximately 130 variants per patient that require further investigation.
In conclusion, causal variant V4 occurs in a high fraction of Belgian STGD1 patients and represents the first deep intronic founder mutation in ABCA4. This emphasizes the importance of augmented molecular genetic testing of ABCA4 in STGD1. Finally, resequencing of the complete ABCA4 gene in the remainder of the STGD1 patients will allow us to identify novel deep intronic and non-coding mutations.

PM02.58
Association between telomere length and telomerase polymorphisms in young adults with cataract.

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INTRODUCTION: Telomeres cells shortening during aging suggests that telomere length could be a biomarker of aging and age-relate morbility. Telomerase compensates this shortening maintaining telomere ends by addition of desoxyribonucleic acid (DNA). Acquired cataract is one of the most common age-related eye diseases but it is also present in a reduced number of young people.
OBJETIVES: The aim of this report was to analyze the prognostic value of telomere length in young patients with cataracts and evaluate the effect of polymorphisms in telomerase genes on the susceptibility to develop cataract in these subjects.
PATIENTS AND METHODS: Telomere length was measured by quantitative polymerase chain reaction in peripheral blood leukocytes of 112 cases and 112 controls classified in two groups (60 years).
To detect telomerase polymorphisms TERT1327C>T and TERC63G>A, TaqMan quantitave PCR method was used.
RESULTS: Our results indicate that young population with cataracts has shorter telomeres than the same cohort of healthy subjects ($p<0.01$) and similar to the older patients with cataracts. The onset of cataracts in younger adults may serve as an in vivo marker of primary aging and at the same time could alert us to other pathologies associated with telomeric shortening as cardiovascular diseases or cancer.
We found a statistically significant association between GG homozygous of TERC63G>A rs2293607 polymorphism and an increased telomere shortening in healthy subjects ($p=0.015$).
Thus, our results suggest that the development of cataracts in young people could be associated to a premature molecular aging.
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PS02.59
Profile of TMPRSS3 mutation among Polish patients with non-syndromic hearing impairment

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Introduction: Recessive mutations of the TMPRSS3 gene cause non-syndromic hearing impairment (HI) but knowledge on their spectrum in Caucasians is limited. The purpose was to search for pathogenic TMPRSS3 variants in Polish Caucasian HI patients.
Materials and Methods: A strategy of iterative cycles of TMPRSS3 gene sequencing (initiated by whole exome sequencing in one subject performed on HiSeq 1500- Illumina) combined with focused mutation screening which we termed Iterative Sequencing and Variant Screening (ISVS) was applied to ~2200 unrelated HI patients recruited among patients from Institute of Physiology and Pathology of Hearing. Approx. 600 control subjects were also studied.
Results: We found 43 (1.88%) probands with 14 different rare TMPRSS3 variants. We also found a genotype-phenotype correlation with truncating mutations causing more severe HI with earlier onset than missense mutations. The p.A90T variant previously linked with HI had high prevalence in controls (~6%) indicating that is non-pathogenic.
Conclusions: TMPRSS3 mutations are rare but distinct cause of HI in Polish population. The more severe phenotype associated with truncating mutations confirms previous findings.
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PM02.60
Editing of the USH2A gene based on CRISPR/Cas9 system

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Introduction: Usher Syndrome is a rare autosomal recessive disease causing sensorineural hearing loss, retinitis pigmentosa and, sometimes, vestibular dysfunction. It is a genetically heterogeneous disorder, with more than 10 associated genes, being *USH2A* the most common mutated gene.
The recent CRISPR technology is based on a nuclease (Cas9) that cuts the double DNA strand at a specific locus via a guide RNA. Upon cleavage, the target locus undergoes a damage repair either by the error-prone nonhomologous end-joining (NHEJ) or the high fidelity homologous recombination (HDR) pathway.
Materials and Methods: We have generated different constructs of CRISPR/Cas9 RNA-guided nucleases targeted to two prevalent mutations located at exon 13 of the *USH2A* gene: c.2999delG and p.C759F. These constructs were transfected into HEK293 cells, and each nuclease efficiency was tested by sequencing and also with the T7 endonuclease assay.
Later on, we repeated the transfection with the most efficient nuclease together with a template to induce HDR repair.
Results: The present study shows the first steps of an approach of gene therapy based on this CRISPR technology, applied to two characteristic mutations responsible for Usher Syndrome.
Conclusions: The pathological features of this syndrome represent a great disadvantage for the patients, since communication nowadays relies largely on audiovisual media, and there is still no medical treatment, except for the hearing aids or cochlear implants for the hearing impairment.
Therefore, repairing of specific mutations by gene editing is an interesting strategy that can be performed using the CRISPR technology.
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PS02.61
A targeted next-generation sequencing diagnostic panel for Usher Syndrome

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Introduction: Usher syndrome is a phenotypically and genotypically heterogeneous autosomal recessive condition comprising both hearing loss and Retinitis Pigmentosa (RP). We have designed a targeted next-generation sequencing panel for the thirteen genes currently associated with Usher syndrome; *ABHD12, CDH23, CIB2, CLRN1, DFNB31, GPR98, HARS, MYO7A, PCDH15, PDZD7, USH1C, USH1G* and *USH2A*. *USH2A* is the most common cause of type II Usher syndrome and is also a common cause of non-syndromic RP.
Method: The genes associated with Usher syndrome are captured using Agilent's Haloplex enrichment technology and sequenced on an Illumina MiSeq. A custom bioinformatics pipeline has been designed for alignment, variant calling, annotation and filtering of variants.
Results: To date 16 patients have been referred with a clinical diagnosis of Usher syndrome. The majority, 11 patients, have two pathogenic USH2A variants – including 3 dosage variants. A further 2 patients have only 1 pathogenic *USH2A* variant. Pathogenic variants were also detected in *MYO7A* and *GPR98*.
USH2A is also tested as part of our Retinitis Pigmentosa diagnostic screen and 10/89 RP patients were found to have two pathogenic *USH2A* variants.
Conclusions: A molecular diagnosis of Usher syndrome was made in 81%

of Usher referrals; 69% in the *USH2A* gene and of these 13% have a copy number variants; confirmed by MLPA. *USH2A* variants are also present in 11% of our RP cohort.

PM02.62
Molecular genetic examination of hereditary deafblindness in Czech patients

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Deafblindness is a rare disorder characterized by combination of hearing and visual impairment. Usher and Stickler syndromes are the most frequent genetic syndromes associated with deafblindness. Usher syndrome is an autosomal recessive disorder responsible for up to 50% cases of hereditary deafblindness. It is divided into three clinical subtypes and is caused by mutations in 11 genes, most frequently in USH2A gene. Stickler syndrome is an autosomal dominant disorder of connective tissues characterized by hearing impairment, eye abnormalities, with possibility of other congenital defects. Mutations in collagen genes, most frequently in COL2A1, are responsible for Stickler syndrome.
In our study we investigated 30 patients from 16 families with Usher syndrome and 17 patients from 9 families with Stickler syndrome from the Czech Republic. We analysed USH2A and COL2A1 genes using Sanger sequencing as a prescreening and we detected mutations in 2 families with Usher syndrome and 2 families with Stickler syndrome. NGS screening of negative patients will follow.
Here we present two cases: The first case is a 50-years-old male with bilateral hearing loss and retinitis pigmentosa. Double heterozygosity of known pathogenic mutation c.11864G>A (Trp3955Ter) and novel mutation c.14621C>G (Ser4874Ter) in USH2A gene confirmed the diagnosis of Usher syndrome in this patient. The second case is a 20-years-old female showing bilateral retinal detachment, experienced by her father and paternal grandfather as well. Identification of a known pathogenic mutation c.1597C>T (Arg533Ter) confirmed the diagnosis of Stickler syndrome.
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PS03.01
17q12 microdeletion - a highly variable syndrome with intrafamilial variation

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Introduction: Congenital anomalies of the kidney and urinary tract (CAKUT) are some of the most common malformations observed during pregnancy. We describe a case of prenatally detected fetal hyper-echogenic kidneys that led to diagnosis of a familial condition.
Materials and Methods: A 34 year old primipara was referred to our institution due to fetal hyperechogenic kidneys at 24 weeks gestation. Her husband has mature-onset diabetes of the young (MODY).
Results: Non-invasive prenatal testing (NIPT) showed low risk for aneuploidy. No other malformations were seen. Fetal ultrasound and MRI were highly suggestive of bilateral renal dysplasia. Amniocentesis with chromosomal microarray analysis (CMA) revealed a 1.5Mb microdeletion on chromosome 17q12 including the TCF2/HNF1B gene. Chromosome 17q12 microdeletion is a well described contiguous-gene deletion syndrome, with variable phenotype. The deletion varies in length but most cases harbor the common 1.5Mb deletion. The TCF2/HNF1B gene is the major causative gene. Features include a wide range of manifestations including neurological, skeletal, hair & nails and endocrine (including MODY). Renal involvement is the hallmark feature, described in over 90% of cases ranging from fetal hyperechogenic or multicystic dysplastic kidneys to pelvic dilatation, recurrent infections and abnormal renal function. Normal kidneys have been described. Parental CMA analysis demonstrated that the deletion was inherited from the father, thus providing a molecular basis for MODY.
Conclusions: Genetic consultation in such prenatal settings is difficult, due to the variable phenotype and penetrance, especially the risk for neuro-developmental disorders, and the fact that it is often inherited from a mildly affected parent.

PM03.02
Phenotypic expansion of visceral myopathy associated with ACTG2 tandem base substitution

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Introduction: Familial visceral myopathy (FVM) is a rare heritable and heterogeneous condition due to impaired smooth muscle function. We identified a family segregating eleven individuals with a spectrum of visceral symptoms involving the small intestine, colon, biliary tract, urinary tract and uterus.
Material and Methods: Whole exome sequencing was conducted on DNA samples from four affected individuals. Target enrichment was performed using the Agilent SureSelect Human All Exon 50Mb kit. Samples were loaded on an Ion PI chip and sequenced on the Ion Proton System using Ion PI Sequencing 200 Kit (200 bp read length
Results: Whole exome sequencing revealed a novel heterozygous tandem base substitution c.806_807delinsAA (p.Gly269Glu) in ACTG2, encoding smooth muscle actin gamma-2, in affected family members.
Conclusions: Variants in ACTG2 were recently identified in familial visceral myopathy with intestinal pseudo-obstruction as well as with the congenital megacystics-microcolon-intestinal hypoperistalsis (MMIH) syndrome. In our family, eight affected members presented with severe complications from the biliary and/or the urinary tracts in addition to gastrointestinal pseudo-obstructions. Furthermore, all affected mothers had a history of assisted deliveries due to poor progress during labor and weak uterine contractions. The variable involvement of multiple smooth muscle dependent organs in our family, including the biliary tract and the uterus, add to the phenotypic spectrum associated with ACTG2 missense variants.

PS03.03
Novel heterozygous mutation in COL4A3 gene in a family with Alport syndrome

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Introduction
Alport syndrome (AS) is an inherited renal disorder characterized by hematuria, progressive renal failure, hearing loss, and ocular abnormalities. The disease is genetically heterogeneous and associated with mutations in type IV collagen that forms a distinct network in the glomerular basement membrane (GBM). Fifteen percent of patients with AS have autosomal recessive inheritance caused by pathogenic mutations in either COL4A3 or COL4A4 genes, located in 2q36-37.
Subjects and methods
Genetic analysis was performed on a 28-year-old male patient who had, since the age of 3 years-old, presented nephrotic syndrome and persistent microhematuria. He fulfilled four of the AS clinical diagnostic criteria: a positive family history of hematuria, proteinuria, hearing loss, and typical electron microscopy abnormalities of the GBM. The entire coding sequence and flanking intronic regions of the COL4A3/A4 genes were analyzed by PCR and direct sequencing reaction. Afterwards, sixteen members of his family (8 males and 8 females) were included in the analysis.
Results
A novel heterozygous mutation (c. [998G>A]+[=]; p.[G333E]+[=]) in exon 18 of the COL4A3 gene was found in the proband and it was confirmed in eleven members of the family.
Conclusion
Among relatives of members who carried the novel mutation, the clinical phenotype of AS was variable. All of them presented hematuria, and 6 of 12 also had proteinuria and hearing loss. The identification of new mutations and their correlation with clinical phenotype are important to offer an early treatment and genetic counselling.

PM03.04
Further evidence for digenic inheritance in Alport syndrome: combined mutations in collagen IV and slit diaphragm genes

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Through a collaborative effort of several centers in Europe, we have recently demonstrated digenic inheritance in Alport syndrome identifying combined mutations in collagen IV genes (Mencarelli et al J Med Genet 2015). This result was achieved using an NGS panel covering COL4A3, COL4A4, and COL4A5 genes. Here, we further expand the evidence for digenic inheritance using an exome sequencing approach. Alport syndrome is characterized by extreme intrafamilial phenotypic variability including the degree of proteinuria. The phenotype of heterozygotes for a single mutation in collagen IV ranges from asymptomatic to overt disease. We selected patients with clinical evidence of Alport syndrome and typical ultrastructural lesions such as thinning, thickening and splitting of the glomerular basement membrane. We demonstrated that symptomatic cases with a single mutation in a collagen IV gene are compound heterozygous at either NPHS1 or NPHS2 locus, with at least one allele being hypomorphic. This combination is not present in male Alport patients hemizygous for one pathogenic mutation in COL4A5. According to the strength of NPHS1/NPHS2 mutations, patients manifest variable grade of proteinuria. In order to prove the pathogenic role of identified mutations we are currently using a method recently described (Lazzeri et al. JASN 2015) which allows isolation and long-term culture of renal progenitors from fresh urine samples. Overall, on the basis of these results, we can further extend the concept of digenic disease for Alport syndrome which can thus be due to either mutations in two collagen IV genes or mutations in one collagen IV gene and one slit diaphragm gene in the framework of triallelic inheritance.

PS03.05

A novel knockin mouse model for Alport Syndrome

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Alport Syndrome represents an inherited nephropathy leading to end-stage renal disease by the 2nd-3rd decade of life. It is characterized by alterations of the glomerular basement membrane (GBM) and is caused by mutations in collagen IV genes. Although most of the responsible mutations are missense substitutions, a full phenotypic analysis of a knockin mouse model is absent from the literature to date.
We herein present preliminary results from the phenotypic analysis of a mouse model carrying the Col4α3-p.G1332E mutation, which is homologous to the human COL4A3-p.G1334E mutation representing a frequent founder mutation among Cypriots with thin basement membrane nephropathy (TBMN).
Mutant mice on the 129SvJ background do not develop hematuria but appear to develop proteinuria around 17-wk-old. Homozygous mutant mice on mixed genetic background exhibit the characteristic Alport GBM ultrastructural features, namely irregular thickening, lamellation and podocyte foot process effacement. Furthermore, heterozygous mice demonstrate diffusely thin GBM, which is the main ultrastructural finding in patients with TBMN. Interestingly, kidney immunofluorescence studies using antibodies specific for the Col4α3 and Col4α5 chains, demonstrate increased expression of both collagen chains in mutant GBM as compared to age/sex matched controls. This may explain why these knockin mice live much longer compared to the existing knockout model. The mean survival of the mutant, heterozygous and wild type mice is 17, 22 and 31 months respectively. The complete phenotypic analysis will enable further clarification of the pathophysiologic mechanisms of Alport syndrome and TBMN and allow the testing of novel therapeutic interventions.
Project was co-funded by the ERDF and the Republic of Cyprus through the RPF (NEWINFRASTRUCTURE/STRATEGIC/0308/24).

PM03.06

Dysregulation of FOXF1 gene and Pulmonary capillary hemangiomatosis (PCH) manifestations.

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Pulmonary capillary hemangiomatosis (PCH) is a rare disorder that was first reported in 1978, with only seven nonrelated cases having been reported by 1998 and a little more than 100 by 2011.
PCH is characterized by numerous blood vessels that proliferate throughout pulmonary interstitial tissue, blood vessels and airways. PCH is pulmonary hypertension associated.
Congenital PCH has been very rarely described.
We report a newborn died after three days of life with severe neonatal pulmonary hypertension, due to diffuse capillary hemangiomatosis diagnosed post mortem.
He had no additional anomalies or malformations, and no familial occurrence.
High resolution conventional karyotype gave normal results (46,XY).
aCGH discovered a chromosome 16q23.3q24.1 deletion that disrupted the distant *FOXF1* trascriptional enhancer *LINC1081*, mapping 0.3 Mb upstream on 16q24.1.
The deletion was *de novo* and sized about 2.6 Mb.
Recently similar deletions of distant *FOXF1* enhacer associated with Alveolar Capillary Dysplasia with Misalignment of Pulmonary Veins (ACDMPV) have been described.
*FOXF1*gene is known to have a crucial role in human lung and intrinsic pulmonary vascular development.
Here we suggest that dysregulation of *FOXF1* expression due to decrease *LINC01081* expression can contribute to histologically proven pulmonary capillary hemangiomatosis, strengthening the hypothesis of a genetic background of PCH.

PS03.07

Characterization of PKD1/PKD2 mutation negative autosomal dominant polycystic kidney disease family

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Autosomal Dominant Polycystic Kidney Disease (ADPKD) is a common (frequency 1: 1000), adult onset nephropathy accounting for 4-10% of patients requiring dialysis or renal transplant worldwide. Most frequently germline mutations in either PKD1 or PKD2 cause ADPKD. However, not only the identification of large ADPKD families unlinked to either gene, but also the lack of PKD1 or PKD2 mutations detected in ~8% of ADPKD families, suggests further genetic heterogeneity. Until now no additional gene has been identified.
We identified a large three generation family with a classical presentation of ADPKD. DNA was available from eight affected and three unaffected relatives. Mutation analysis of PKD1 and PKD2 ruled out a deleterious mutation in these genes; furthermore, linkage analysis ruled out an association with these loci. Genome-wide SNP analysis suggested linkage with a locus of 5 Mb on chromosome 19 (LOD score= 2.3994). Exome sequencing did not reveal a clear deleterious mutation in this family in this region. Since the coverage of several top candidate genes was not sufficient, we are currently screening the coding regions of these genes by next generation sequencing. Further molecular exploration of this family may lead to the identification of a novel gene associated with ADPKD.

PM03.08

PKD2-related Autosomal-dominant Polycystic Kidney Disease (ADPKD): mutation spectrum, phenotype and prognosis

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PKD1 and PKD2 mutations are identified in ~85% and ~15% of the Autoso-

mal-dominant Polycystic Kidney Disease (ADPKD) pedigrees. As targeted therapies are emerging, accurate description of ADPKD phenotype is crucial to delineate which patients should, or should not, receive these new treatments. PKD2 phenotype has been described as milder but population-based studies, enabling to depict the exact burden of the disease, are currently lacking. We aimed to describe PKD2 mutation spectrum and related phenotype. Between 2010 and 2015, a mutation of PKD2 was identified in 241 patients from the cross-sectional Genkyst study (166 pedigrees).
The 68 different mutations identified included 23 newly described mutations. They spanned on the entire coding region of PKD2 gene and were mainly truncating mutations (84%). A 28-kb deletion involving exons 10-15 was identified in 23 pedigrees confined in an area of ~3500 km^2, which represents the first case of founder mutation in ADPKD. At inclusion, 50 patients had reached end-stage renal disease (ESRD), and median age at ESRD (obtained by Kaplan-Meier model) was 77.8 yrs [ranges=41.5-84.6]. Although there was no gender influence on renal survival, multiple linear regression demonstrated that men had significantly lower kidney function than women. Considerable disease variability was observed amongst patients harbouring the same mutation, suggesting that additional genetic or environmental factors may modulate the disease severity in PKD2 patients. Consistent with this point, in 3 PKD2 pedigrees with cases of early-onset ESRD, disease severity was linked to the co-inheritance of hypomorphic alleles of PKD1. (funded by PHRC inter-regional 2010)

PS03.09
Genomic analysis of biliary atresia by GWAS and whole exome sequencing

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Biliary atresia (BA) is a rare liver disease presenting within the first months of life characterized by obliteration of the extrahepatic biliary tree in a progressive, necroinflammatory manner, leading to cholestasis, fibrosis, cirrhosis and chronic liver damage, and accounting for 50% of pediatric liver transplantations in the US. The etiology of BA is not well understood, and environmental, inflammatory, and genetic causes have been proposed. A GWAS in Chinese patients previously identified a signal on chromosome 10q upstream of the ADD3 and XPNPEP1 genes. We performed a GWAS in 450 Caucasian non-syndromic BA patients collected through the Childhood Liver Disease Research Network and 1981 controls, with the Illumina Omni2.5 array. The most significant SNP was rs10865291 (p-value = 2.7x10-7; odds ratio 1.6; 95% CI (1.3 -1.9)), in the sixth intron of EFEMP1. Efemp1 is an extracellular glycoprotein implicated in tissue regeneration and organogenesis. Additionally, we sequenced the exome of 100 patients, and looked for rare variants in 21 genes chosen because of reports of mutations in syndromic BA or animal models with hepatobiliary defects, and BA association from GWAS. Filtering for protein-altering variants present at <1% in public datasets, we found 20 missense variants and 2 in-frame insertions in 13 different genes in 26 patients. We found more than one variant in LGR4, ZEB2, DNMT1, INVS, JAG1, and PRICKLE4, some shared among more than one proband. These findings suggest that BA etiology is likely highly heterogeneous and a single gene does not explain the phenotypic complexity of the disease.

PM03.10
Prioritization of candidate variants using targeted next generation sequencing of 208 candidate genes in congenital anomalies of the kidney and urinary tract

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Introduction: The leading cause of end-stage renal disease in children is attributed to congenital anomalies of the kidney and the urinary tract (CAKUT). Familial clustering implicate genetic factors and monogenic mouse models support the involvement of numerous genes in CAKUT aetiology. However, genetic testing for human CAKUT is currently insufficient and additional efforts are needed to identify causal genes.
Materials and Methods: We designed a gene panel of 208 genes, which consists of known genes identified from studies on familial forms of isolated or syndromic CAKUT and candidate genes suggested by in vitro and in vivo models for CAKUT. Targeted next-generation sequencing was performed in 458 patients with sporadic CAKUT with a depth of coverage of 135x per sample.
Results: Rare (frequency < 0.01), truncating, splice-site variants and non-synonymous variants predicted to be deleterious and conserved were defined as candidates. Based on previous reports on disease-causing mutations, we considered 5 variants in 6 patients to be causal mutations and 15 variants in 20 patients to be likely pathogenic. However, 32 variants found in 69 patients that were previously reported as pathogenic were reclassified here as variants of uncertain significance. Finally, we identified 128 candidate variants in 132 patients that were predicted to be loss-of-function.
Conclusions: This study comprises the largest gene set to be analyzed in a cohort of CAKUT patients to date, it demonstrates the advantages of a disease-targeted gene panel, and prioritizes candidate variants identified in 34% of our patient cohort, some of which are currently being followed up using in vitro functional tests.

PS03.11
Whole exome sequencing and targeted sequence analysis in 73 patients with congenital anomalies of the kidneys and urinary tract (CAKUT)

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CAKUT is a genetically highly heterogeneous disorder. To elucidate the genetic aetiology in unclear cases and identify novel CAKUT genes, we performed whole exome sequencing on peripheral blood from 24 patients (22 sporadic, 2 familial) followed by detailed data analysis. In 12/24 (50%) patients, we found 14 different heterozygous loss-of-function (LOF) variants in two known dominant CAKUT genes (PAX2 und SALL1) and 11 CAKUT candidate genes, whereby two patients carried two distinct LOF mutations. Four of the 14 LOF variants were de novo, one was inherited from an affected parent, seven were inherited from a healthy parent, and in two cases inheritance was undetermined. In four patients, known CAKUT causing missense mutations inherited from a healthy parent were detected in the SIX2 (2/24) and the RET (2/24) gene. In 8 patients without LOF variants, heterozygous missense variants were determined in CAKUT candidate genes that were novel and predicted to be pathogenic, three of which belonged to the GDNF-RET-network. In 49 additional CAKUT patients, targeted sequencing of selected candidate genes was performed. One of these genes, in which we detected four rare or novel heterozygous variants including one de novo LOF and three pathogenic missense variants in CAKUT patients, was further analysed in vitro and in vivo. We characterised its expression pattern in mouse embryos during the development of the kidney and urinary tract by RNA in situ hybridisation. In addition, we examined the kidneys and urinary tract of mice with a knock-out of this gene macroscopically and microscopically.

PM03.12
The first genetic study on congenital choledochal dilatation (CCD) implicates extracellular matrix proteins

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Congenital choledochal dilatation (CCD) or paediatric choledochal cyst refers to the congenital dilatation of the choledochs (bile ducts) which leads to the obstruction of the ducts and bile retention. Symptoms include cholestatic jaundice, abdominal pain and liver enlargement complicated with cholangitis and pancreatitis. New-borns undergo surgery otherwise the liver could be permanently damaged. CCD is rare, mostly sporadic with variable population incidence, the highest being in Asia (1/1,000 in Asians; 1/150,000 in Caucasians). Its aetiology implicates congenital structural anomalies reflecting a failure in the hepatobiliary-pancreatic development.
Thirty-one CCD trios were exome sequenced. Gene/pathway-set enrichment

analyses grouped genes with at least one damaging allele into focal adhesion and extracellular matrix-receptor interaction pathways.
Pathogenic mechanisms considered included *de novo* germ-line mutations and/or recessive inherited mutations in homozygosis, compound heterozygosis (CH) or as "di-genic/oligogenic" model of inheritance whereby variants in genes of related pathways coexist in a patient through parental inheritance.
Fifteen gene members of those pathways were recurrently mutated and had variants at different sites (more than one damaging allele per gene). These alleles were in compound heterozygosis or co-existing with a mutated functional gene-partner in the same individual.
Patients' genetic profiling revealed CCD as not only genetically heterogeneous but with di/oligogenic inheritance. Yet, the relevant mutated genes are functionally convergent. Data are consistent with the sporadic presentation of CCD. Incidentally, the cholangiocarcinoma rate in Asians is also the highest world-wide. We
are also aiming at finding possible links between these choledochal disorders and at explaining their high incidence in Asia.
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PS03.13
Report of two CTRC intronic mutations associated with acute or chronic pancreatitis and delineation of their pathogenic molecular mechanisms

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Chymotrypsin C (CTRC) is highly specific in degrading all human trypsin/trypsinogen isoforms, constituting an important line of defense against prematurely activated trypsin within the pancreas. Since 2008, variants in the CTRC gene have been increasingly reported to be associated with chronic pancreatitis. However, to date, all reported CTRC intronic variants have been classified as "unknown significance" due to the lack of functional analytic data. During our routine mutational analysis of four pancreatitis genes, PRSS1, SPINK1, CTRC and CFTR, in patients with acute or chronic pancreatitis, we identified two intronic mutations in the CTRC gene, c.357-2A>G in intron 4 and c.640-12G>A in intron 6 (Table), both of which were absent in 350 healthy controls. We performed RT-PCR analyses of mRNAs prepared from cultured lymphocytes of patients I and II (Table), respectively: c.357-2A>G activated a cryptic splice acceptor signal that is located 147 bp upstream of the wild-type exon 4 whilst c.640-12G>A created a novel splice acceptor signal 8 bp upstream of the normal one. This is the first report of experimentally confirmed pathogenic CTRC intronic mutations associated with acute or chronic pancreatitis. The identification of a pathogenic CTRC variant in the three children or adolescent patients with acute pancreatitis has important implications for genetic counseling; these patients should be advised to avoid alcohol and smoking because they are at high risk in developing chronic pancreatitis.

Clinical data of pancreatitis patients with CTRC intronic mutations

Patient (sex)	Ethnicity	Diagnosis	Current age (y)	Age of first onset (y)	Family history	Mutation in CTRC	Mutation(s) in other pancreatitis genes
I (female)	French Caucasian	Chronic calcific with diabetes	58	20	Mother with the disease	c.357-2-A>G	CFTR p.F508del and SPINK1 c.27delC
II (male)	Moroccan	Acute	7	4	No	c.640-12G>A	No
III (female)	Cameroonian	Acute recurrent	20	18	No	c.640-12G>A	No
IV (female)	Algerian	Acute recurrent	12	6	No	c.640-12G>A	No

PM03.14
Genetic testing for congenital adrenal hyperplasia: the Canadian experience

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Congenital adrenal hyperplasia (CAH) most commonly results from a deficiency of 21-hydroxylase, an enzyme in the biosynthetic pathway that converts cholesterol to cortisol and aldosterone. Mutations in CYP21A2, encoding 21-hydroxylase, are identified in >80% of individuals with CAH. CYP21A2 and its highly homologous pseudogene, CYP21A1P, reside within close proximity on 6p21; as a result >90% of CYP21A2 mutation-containing alleles are caused by gene conversion events from CYP21A1P or CYP21A2 deletions arising from non-allelic homologous recombination. Further, CYP21A2 full gene duplications exist and provide a source of false negative and false positive results as carriers of a CYP21A2 deletion may be masked by a CYP21A2 duplication on the opposite allele (2+0 configuration) or carriers of a point mutation may also carry a second non-mutated CYP21A2 in cis. In our lab current CYP21A2 testing consists of dosage analysis by MLPA and coding region sequencing from full-length gene PCR amplification, which currently will not detect 2+0 CYP21A2 deletion carriers. Here we present the distribution of mutations detected during genetic testing from the only CYP21A2 testing laboratory in Canada and present additional data further supporting the need for dosage analysis in all CYP21A2 analyses. We also present an experimental approach to identify individuals with 2+0 silent deletions and provide preliminary data of the prevalence of the 2+0 configuration in a control population of >900 individuals.

PS03.15
Molecular genetic diagnostics of congenital adrenal hyperplasia in the Czech Republic

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Background: Congenital adrenal hyperplasia (CAH) is a group of inherited autosomal recessive disorders caused by enzymatic deficiency which impairs steroid hormone biosynthesis. About 90 % of all CAH cases are due to 21-hydroxylase deficiency (21-OHD) caused by *CYP21A2* gene aberrations. Most of the *CYP21A2* gene mutations result from recombination events with its pseudogene *CYP21A1P*. Mutations in the *CYP11B1, HSD3B2* and *CYP17A1* genes are genetic causes of 11-β-hydroxylase deficiency (11-βOHD), 3-β-hydroxysteroid dehydrogenase deficiency (3-βHSD) and 17-α-hydroxylase deficiency (17-αOHD), respectively.
Methods: For differential amplification of the genes and their homologs, long-range PCR is used (21-OHD, 11-βOHD). Diagnostics of 21-OHD is followed by restriction analysis, secondary PCR, sequencing and MLPA. Further *CYP11B1* analysis comprises secondary PCR and sequencing. Molecular diagnostics of 3-βHSD and 17-αOHD include PCR and sequencing.
Results: Molecular genetic testing confirmed the diagnosis of 21-OHD in 373 probands (45,8 %). Only one mutant allele was found in 80 (9,8 %) of the probands. The diagnosis of 11-βOHD was confirmed in one patient and 17-αOHD was confirmed in two patients. Analysis of -1888G/T and -1858A/G *CYP11B1* promoter SNPs revealed 23 probands (29,1 %) homozygous for -1888 T and -1858 G alleles. Homozygosity of these alleles is associated with reduced 11-β-hydroxylase efficiency.
Conclusion: Molecular genetic testing in our laboratory allows for the identification of mutations in the *CYP21A2, CYP11B1, HSD3B2* and *CYP17A1* genes in patients suspected of steroid hormone synthesis deficiency.

PM03.16
Whole Exome Sequencing in Congenital Hypogonadotropic Hypogonadism

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Congenital hypogonadotropic hypogonadism (CHH [MIM161110]) due to gonadotropin-releasing hormone deficiency is a rare genetic disorder (affects ~1/30,000) characterised by abnormal pubertal development and infertility. Over 60% cases have anosmia (Kallman syndrome) and some exhibit additional phenotypes. CHH is a genetically heterogeneous developmental disease. Most cases present sporadically, although familial forms (AD, AR and X-linked) with incomplete penetrance and variable expressivity occur. Research suggests it is emerging as a digenic or oligogenic disease, rather than a monogenic trait. Genetic testing for this condition has hitherto been costly, time-consuming and incomplete, with mutations identified in less than 30%.
In this study, we investigated three simplex cases (2M with partial anosmia, 1F) who presented with delayed puberty and hypogonadism due to isolated GnRH deficiency. Whole exome sequencing (WES) was performed on each patient using the Illumina HiSeq2500 platform and the Agilent SureSelect

Human All Exon v5 Kit. Following alignment to the human genome, an in-house pipeline applied a virtual panel to restrict the genes to be analysed based on the clinical presentation (22 CHH genes). Variants were filtered to identity potential pathogenic mutations, which were subsequently confirmed by Sanger sequencing.
Results: Five variants in three CHH genes (FGFR1, GNRHR and HS6ST1) were identified. Variants in these genes are recognised to result in deficient GnRH activity, confirming that a Kallman phenotype can result from mutations in CHH genes other than KAL1.
Conclusion: We demonstrate that WES, with analysis limited to relevant genes, can successfully and efficiently confirm a molecular diagnosis in a genetically and phenotypically complex disease, such as CHH.

PS03.17

Comprehensive genetic analysis of 35 unrelated Spanish patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Report of a novel frameshift mutation in the CYP21A2 gene.

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We describe the *CYP21A2* mutation spectrum in a cohort of 35 unrelated North-East Spanish congenital adrenal hyperplasia (CAH) patients; 29 women and 6 men, 25 of them diagnosed in pediatric age. The distribution of the phenotypes was: salt-wasting SW (7 patients), simple virilizing SV (5 patients) and non-classical form NC (23 patients). All *CYP21A2* exons were sequenced to determine mutations and MLPA was performed to detect *CYP21A2* large deletions. A genotype-phenotype correlation was investigated. Two mutated alleles were detected in all SW and SV patients as well as in 19 of the 23 NC patients. In the remaining four only one mutated allele was found. A novel p.Leu343Cysfs*20 mutation was identified in a NC patient.
The following table shows *CYAP21A2* allele mutations by phenotype:

	SW	SV	NC	Total
p.Val282Leu			30(71,4%)	30(45,5%)
Total deletion	7(50%)	2(20%)		9(13,6%)
p.Ile173Asn		6(60%)	2(4,8%)	8(12,1%)
IVS2-13 A/C>G	4(29%)		3(7,1%)	7(10,6%)
p.Pro453Ser		1(10%)	2(4,8%)	3(4,5%)
p.Pro483Ser			2(4,8%)	2(3%)
p.Arg484fs	2(14%)			2(3%)
Gene conversions	1(7%)	1(10%)		2(3%)
p.Arg357Trp			1(2,4%)	1(1,5%)
p.Gln319X			1(2,4%)	1(1,5%)
p.Leu343Cysfs*20			1(2,4%)	1(1,%5)
Total	14	10	42	66

The most prevalent mutation in NC patients was p.Val282Leu, whereas p.Ile173Asn and a complete gene deletion were the most observed mutations in SV and SW patients, respectively. These findings are consistent with previously published data of a northern-Spain cohort. Genotype accurately predicted phenotype in more than an 85% of our patients. The finding of CAH cases with only one mutated allele underlies the importance to implement deep-sequencing tests to identify cryptic mutations in *CYP21A2* or mutations in other candidate genes.

PM03.18

Application of next-generation sequencing in the search of causative genes for Taiwanese patients with disorders of sex development

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Objectives
This study aims primarily to establish a rapid and high-throughput genetic test for 46,XY DSD. Meanwhile, the applicability and effectiveness of next-generation sequencing are to be evaluated in searching for the remaining unknown causative genes of this inherited disease.
Methods
A membrane hybridization method was used as the main strategy for target sequence enrichment. In a stepwise manner, the capture filter for target genes were designed and developed under specific conditions so that whole-genome fragment DNA libraries could be set up appropriately. The amplified exomes of target genes were hybridized against the DNA libraries and sequenced subsequently.
Results
We have enrolled 6 46, XY DSD patients in this study. Targeted smplicons have been generated and are currently under investigation of their capture efficiency. Exome sequencing has been performed with genomic DNA from two of the patients. Potential disease-causing variants have been identified. A total of 2 genetic variants were identified and thought as the main cause for DSD. One is a missense mutation F846 on the ATRX gene, while the other one is indel with V176_K177insV on the TSPYL1 gene.
Conclusion
Finding the causative gene of a heterogenetic disease is challenging even with recent development of NGS and bioinformatics. In our study, we have a list of candidate disease-causing variants that need to be carefully studied in the cell model system with biochemical assays. Our results indicate that NGS aids on genetic diagnosis of 46,XY DSD.

PS03.19

RNA-Seq in skeletal muscle of metabolic syndrome patients with DYRK1B_R102C mutation

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Methods: Oral Glucose tolerance test (OGTT) was carried out in non-diabetic young DYRK1BR102C mutation carriers and non-carrier relatives. The skeletal muscle (SM) biopsies of these probands were examined by RNA-Seq and Western blot analysis. In addition, wild type and mutant DYRK1B and empty lentivirus constructs were administrated to C56Bl6 mice and their effects on body weight and plasma glucose and key regulators of insulin signaling were examined.
Results: Pathway-based analysis of the SkM RNA-Seq data revealed preferential expression of genes involved in cytoskeletal remodeling, including ACTN3, SLC16A3, MYH11, Grem2, MYL6B, MSS51, and MYLPF. Consistent with RNA-Seq data, staining of SkM of carriers and non-carriers for slow- and fast-twitch fibers demonstrated higher ratio fast glycolytic fibers in mutation carriers than non-carriers (65% vs. 40%). DYRK1B mutation carriers exhibited higher baseline insulin levels (16.7uIU/mL) and compared to non-carriers (8.4uIU/mL) and had higher plasma glucose levels in response to oral glucose ingestion that is indicative of insulin resistance in carriers of R102C mutation. Mice overexpressing DYRK1BR102C allele were similarly insulin resistant.Western blot analysis and Mass Spectrometry of liver proteins illustrated increased expression of G6Pase, PEPCK, Glucokinase, and Pyruvate carboxylase, in mice overexpressing mutant and wild type DYRK1Bcompared to empty vector.
Conclusions: Our findings suggest that DYRK1B initiates cytoskeletal remodeling and causes insulin resistance, while it triggers hepatic gluconeogenesis by enhancing the expression of key gluconeogenic enzymes.

PM03.20

Genetic and functional interactions between β1 integrins and endothelin-3 during enteric nervous system development

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The enteric nervous system (ENS) results from the colonization of the developing gut by enteric neural crest cells (ENCCs). During their migration, ENCCs proliferate and differentiate into glial and neuronal cells, which aggregate into ganglia and give rise to the intrinsic innervation of the bowel. A proper ENS formation requires many different factors among which are the G-coupled receptor EDNRB, its ligand endothelin-3 (EDN3), and Itgb1 encoding the β1-integrin subunit. Interestingly, the enteric phenotype of conditional Itgb1 mutants resembles the phenotype described for Edn3 or Ednrb mutants, i.e. lack of innervation in the distal part of the colon, reminiscent of Hirschsprung disease in Human. Otherwise, EDN3 was shown to regulate adhesion properties of cancer cells and astrocytes.

Considering these observations, we investigated a putative role of EDN3 on ENCC adhesion properties and its functional interaction with β1-integrins during ENS development. We discovered that EDN3 promotes ENCC adhesion in vitro. It stimulates β1-integrin activation and increases the number of ENCCs focal adhesions. Upon EDN3 treatment, ENCCs rapidly exhibited changes in cell shape and membrane dynamics displaying a sustained growth and persistence of lamellipodia. Morever, in vivo double-mutant studies showed that itgb1-/-; Edn3ls/ls mutants displayed an aggravated enteric phenotype and an altered ENS network organization. Ex-vivo live imaging of embryonic guts allowed us to evidence severe migratory defects of double mutant ENCCs that contribute to the enteric defects observed.
Altogether our results reveal that interplays between EDN3 and β1-integrins are crucial for proper ENS ontogenesis.

PS03.21
The analysis of APOB-100, LRPAP1, ABCG5 and ABCG8 genes polymorphisms in gallstone disease patients and healthy donors from Volga-Ural region of Russia

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Gallstone disease (GSD) is a metabolic diseases of the hepatobiliary system, characterized by the formation of gallstones in the gallbladder, common bile duct stones in the liver bile ducts. 10% of the population suffers from gall stones, and the number of patients in the world with each passing decade becomes larger. The aim of this study was to exam the association of polymorphisms of APOB-100 (rs693), LRPAP1 (rs11267919), ABCG5 (rs4131229) and ABCG8 (rs11887534) genes with the risk of gallstone disease. The patient group consisted of 205 patients with with cholelithiasis, the control group included 190 unrelated healthy individuals. Genomic DNA was extracted from peripheral blood leucocytes by standard phenol/chloroform method. Genotyping was performed by PCR followed by restriction digestion. The analysis has revealed that heterozygous genotype X+X- of rs693 of APOB-100 gene is a marker of increased risk of gallstone disease in Russian (p = 0.03; OR = 2,1). For those of Tatar ethnicity shows that rs693*X-allele and C allele of rs4131229 of ABCG5 gene are markers of increased risk of developing the disease (p = 0,002; OR = 2,0 and p = 0,02; OR = 1,7, respectively), while rs693*X +, rs4131229*T alleles and of rs4131229*T/T genotype are a markers of reduced risk of gallstone disease (p = 0,002; OR = 0,5; p = 0,02; OR = 0.6 and p = 0,03; OR = 0,5, respectively). Results of the study shows that the polymorphisms of the APOB-100 and ABCG5 genes are associated with the risk of gallstone disease.

PM03.22
The mutational analysis of the INF2 gene in Czech patients with FSGS and MCD

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We started to screen for mutations in the INF2 gene in 136 Czech patients (49.82 ± 17.91) with FSGS/MCD. The INF2 protein plays a key role in the function of the slit diaphragm in podocytes.
We have so far identified one already known missense mutation with the proven damaging effect on the function of podocytes. The substitution Arg218Gln, which was in the heterozygous state, was found in two brothers (thirty-two and thirty-one years old) with a positive family history who suffered from FSGS. The heterozygous state did not correlate with the rapid progression and early development of the ESRD (twenty-seven and thirty-one years, respectively), while their father developed ESRD at fifty-seven years. For that reason, we suppose the collaboration of more factors, such as other substitutions in the INF2 gene or in other genes connected with FSGS or the influence of environment, in the case of these two brothers.
The other interesting findings were the undescribed heterozygous changes p.Pro208Ser (c.622 C>T) in the anonymous patient and p.Pro1057Leu (c.2640 C>T) in forty-three years old man (FSGS, negative family history). However, according to predictive programs (PolyPhen-2 and PON-P2), this substitution is not probably causal. It was also found the already known substitution p.Arg214His (c.641 G>A) with proven damaging effect in fifty-one years old woman (FSGS, positive family history).
The previous mentioned results are first data from the mutational analysis of the INF2 gene in Czech patients with FSGS/MCD. The similar study focused on the INF2 gene has never been performed in the Czech Republic.
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PS03.23
Abernathy malformation: a rare association with Goldenhar syndrome

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Abernathy malformation is a rare congenital malformation characterised by absence or hypoplasia of the hepatic portal vein, resulting in a congenital portosystemic shunt. Consequences of the malformation include focal nodular hyperplasia of the liver, hepatoblastoma and hepatic encephalopathy.
Our patient is a 27 year old woman who was referred to the genetics clinic for preconception counselling due to a diagnosis of Goldenhar syndrome. She was diagnosed with focal nodular hyperplasia of the liver aged 15. Review of historic imaging and magnetic resonance cholangiopancreatogram revealed a type 1a Abernathy malformation, likely to be the cause of the focal nodular hyperplasia.
Congenital absence of the portal vein has been previously described in one individual with Goldenhar syndrome. In this case focal nodular hyperplasia developed into hepatoblastoma requiring liver transplantation. Abernathy malformation is a rarely reported association of Goldenhar syndrome that can cause serious hepatic complications.

PM03.24
Metallothioneins are downregulated in ileal mucosa of Familial GUCY2C diarrhea syndrome patients susceptible to Crohn´s disease

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Background: Familial *GUCY2C* diarrhea syndrome (FGDS), first described in a Norwegian family (n=38), is caused by an activating mutation in the gene encoding guanylate cyclase C. Patients with FGDS have early onset mild diarrhea but are also susceptible to ileal Crohn's disease (CD) (7 patients). The aim of the present study was to compare global gene expression in ileal biopsies (non-inflamed mucosa) from FGDS patients (n=11), unrelated CD patients (n=6) and healthy controls (n=16). We also assessed whether CD genetic risk variants segregate with CD in the FGDS patients.
Methods: Global gene expression was examined using Illumina Human HT-12 v4 BeadChip. 140 CD risk variants were genotyped (Immunochip array) and the *NOD2* gene was sequenced in 23 adult FGDS-patients (7 with CD).
Results: Nine metallothioneins were significantly downregulated (1.5-3 fold) in FGDS patients, but not in unrelated CD patients, compared to controls. The polygenic risk score did not differ significantly between FGDS patients with and without CD. However 6 of the 7 FGDS patients with CD carried *NOD2* risk variants, and the two most severely affected patients were homozygous for the rs5743289 risk allele . Three of 16 FGDS patients without CD were heterozygous for *NOD2* risk variants
Conclusion: Metallothioneins were significantly downregulated in non-inflamed terminal ileum of FGDS patients, but not in unrelated CD patients compared to controls. Lower levels of these zinc-binding proteins may cause inflammation due to interference with NOD2-stimulated bacterial clearance and autophagy. Further studies are warranted to investigate guanylate cyclase C-related susceptibility to Crohn´s disease.

PS03.25
Molecular analysis of KAL-1 and GnRHR genes in patients with idiopathic hypogonadotropic hypogonadism

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Introduction: Idiopathic hypogonadotropic hypogonadism (IHH) comprises delayed/absent puberty, infertility and low serum gonadotropins in the context of normal anterior pituitary anatomy and function. Is due to partial/complete absence of gonadotropin-releasing hormone release/action or gonadotropin secretion and its incidence is low (1/10.000-1/86.000) with a nearly 4:1 male-to-female ratio. Approximately two-thirds of individuals with IHH have anosmia or hyposmia (Kallmann syndrome-KS) and one-third

have normosmic IHH (nIHH). Mutations in *KAL1* gene cause X-linked KS, and in *GnRHR* gene autosomal recessive IHH (almost 2% of nIHH patients).
Material and Methods: 45 patients with IHH (42 males, 3 females), with and without hyposmia/anosmia were studied. Mutation analysis of *KAL1* and/or *GnRHR* was performed by SSCP/DHPLC-PCR or by PCR-direct DNA sequencing.
Results: We found two *KAL1* mutations: c.769C>T (p.Arg257*) in a 15-years-old anosmic male; and a novel one, an extensive deletion encompassing exons 4 to 14 confirmed by MLPA, in a 3-years-old boy (detected also in his mother) with micropenis and maternal family history of HH. Two nIHH male patients were compound heterozygous for *GnRHR*: c.[2T>C];[785G>A], p.[(M1T)];[(R262Q)] (39-years-old, prepubertal testicules, gynecomastia, P1-A0 pilosity) and c.[317A>G];[416G>A], p.[(Q106R);(R139H)] (35-years-old with a brother non-tested with similar phenotype). All four mutations are known to be disease-causative.
Discussion: We were able to find the genetic defects in 4 patients. The low detection rate of mutations (8.8%) is related with the existence of several genes implicated in the IHH' pathogenesis. The NGS analysis in patients with IHH may improve the molecular diagnosis as it allows the screening of different genes simultaneously.

PM03.26

Systematic analysis of chromatin interactions at disease associated loci links novel candidate genes to Inflammatory Bowel Disease

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Introduction: Genome wide association studies (GWASs) have revealed numerous genomic loci that are associated with complex genetic diseases. Subsequently, many candidate genes have been defined, mainly based on the functional relationships between genes found in the vicinity of the identified loci. However, many of these loci can be linked to regulatory DNA sequences and it is now widely appreciated that part of the GWAS associations is due to sequence variation in regulatory elements. Therefore, the genes controlled by these regulatory elements should be considered as possible candidate genes. Since regulatory elements can regulate genes via chromatin-chromatin interactions that comprise up to 1 Mb, these genes cannot be identified based on base-pair distance from the regulatory regions. To address this, we used chromatin conformation capture-sequencing (4C-seq) to systematically determine the genes that are physically interacting with regulatory units that overlap the disease associated SNPs in Inflammatory Bowel Disease (IBD).
Results: We assayed chromatin interactions in monocytes, lymphocytes and in DLD-1 cells – major cell types implicated in IBD pathogenesis. We performed 4C-seq for 92 IBD-associated loci that localize to regulatory elements in all three cell types. Our approach links 815 novel genes, including *IL10RA, SMAD5, SMAD6* and *PIAS1*, to IBD.
Conclusion: We have performed a novel candidate gene approach in which chromatin interaction data on GWAS-susceptibility loci are intersected with the information about DNA regulatory elements and gene expression in relevant cell types. This revealed 815 novel candidate genes, consisting of multiple notable genes like *SMAD6, IL10RA, PIAS1* and *SMAD5,* thereby complementing previously reported candidate gene approaches.

PS03.27

Johanson-Blizzard syndrome in an Omani infant with neural tube defect: a coincidental findings or a consequence of UBR1 mutation ?

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Johanson-Blizzard syndrome (JBS), #243800 is a rare, autosomal recessive multisystem disorder characterized by exocrine pancreatic insufficiency and aplasia/hypoplasia of alae nasi. Additional common features include ectodermal dysplasia, hypothyroidism, growth hormone deficiency, sensorineural hearing loss, urogenital and anorectal anomalies and cognitive dysfunction of variable degrees. Mutations of *UBR1* (MIM #605981) are known to cause JBS. The UBR1 represents one of at least four E3 ubiquitin ligases of the N-end rule pathway, an evolutionary conserved and ubiquitously expressed intracellular proteolytic pathway involved in ubiquitin-mediated degradation of many proteins. JBS has wide and highly variable clinical manifestation with rare malformations observed in some patients with molecularly confirmed JBS. We report a newborn Omani with JBS and a novel truncating mutation in *UBR1*. The clinical features include a beaked nose, hypoplasia of nasal wings, exocrine pancreatic insufficiency presenting with severe failure to thrive and septicemic shock, severe anemia requiring frequent blood transfusion, anal atresia, sparse hair, scalp defect, lumbosacral meningo-myelocele and hydrocephalus.This is a second report of neural tube defect in association with JBS implying that this association is as a result of *UBRI* mutation rather than coincidental. The phenotypic defects in JBS involve several organ systems in addition to pancreas suggesting that UBR1-mediated protein degradation plays a critical role at certain stages of human development, and in specific cell types.

PM03.28

Targeted panel sequencing of 399 renal genes reclassifies primary disease diagnoses in young end stage renal disease patients

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Background: About a quarter of patients with end stage renal disease (ESRD) before age 30 do not have a primary renal disease diagnosis. Previous genetic studies have focused on specific clinical diagnoses. We took an innovative approach by sequencing a panel of 399 renal disease genes in 200 cases with ESRD onset before age 30, regardless of their clinical diagnosis. Data for the first 132 cases are presented.
Methods: We designed the "RENome" using SureSelect/Agilent with 399 genes involved in hereditary renal disease. We used SOLiD™ 5500XL for sequencing and an in-house developed bioinformatics pipeline for mapping, variant calling and QC. Variants were annotated using CARTAGENIA software.
Results: On average >95% of in target bases were genotyped, with >99% sensitivity and specificity. Stringent filtering criteria allowed only for coding variants with percentage variant reads of >15%, novel or with allele frequency of <0.005, that were listed as disease-causing in HGMD Pro, had a SIFT score <0.05 and were not predicted to be benign in PolyPhen2. We also selected samples with likely CNVs. Extended analyses, with less stringent filtering criteria and in depth copy number analyses, are presented at the meeting.
Conclusion: This filtering strategy yielded a molecular diagnosis in 15 patients (11.4%), confirming the registered primary disease in 6, and unexpectedly reclassifying it in 9. Considering the stringency of filtering, these numbers underestimate the diagnostic potential of our innovative approach. Adding early RENome sequencing to the diagnostic work-up in all young ESRD patients, improves etiologic classification and genetic counseling.
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PS03.29

Significant association of KIR2DL3/HLA-C1 combination with susceptibility to Crohn´s disease

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Introduction: The killer cell immunoglobulin-like receptors (KIRs) form a group of regulatory molecules that specifically recognize HLA class I molecules. The aim of this study was to analyze the possible association of specific KIR genes and KIR/HLA-C genotypes with the susceptibility to Crohn's disease (CD) in a Spanish population.
Materials and Methods: A total of 125 patients with RA and 339 healthy control subjects were selected for this study based on clinical criteria. The commercial KIR-SSO typing kit from Luminex (Tepnel Lifecodes) was used to investigate KIR and HLA-C typing.
Results: The centromeric A/A genotype was more frequent in CD patients ($P<10^{-3}$). When we included HLA-C analysis, we found that the centromeric A/A genotype and HLA-C1 combination was significantly increased in CD patients ($P<10^{-3}$). Moreover, KIR2DL2/2DL3 genotype demonstrated a decreased frequency in CD patients (P<0.0005), whereas the KIR2DL3/2DL3 genotype was significantly increased in CD patients (P<0.0005). Remarkably, we also observed a highly significant increase of the KIR2DL3/KIR2DL3 HLAC1/HLAC1 homozygosis in CD patients (P<0.0005).
Conclusion: Our results confirm the relevance of the KIR2DL2/KIR2DL3 genes and their interaction with HLA-C in CD. The presence of a particular KIR-HLA pair may confer functional competence on NK cells and influences differences in NK cell functional responses among individuals. We show that the contribution of the KIR genes to CD susceptibility extends beyond the association with individual KIRs, with an imbalance between activating and inhibitory KIR genes seeming to influence the susceptibility to CD.

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PM03.30

Endocrine neoplasia: translational outcome of the genetic study

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Multiple endocrine neoplasia (MEN) syndromes are characterized by tumors of endocrine and non endocrine organs. They are autosomal dominant disorders and categorized into MEN1, MEN2, Carney complex (CNC), von Hippel-Lindau disease (VHL) etc. caused by genetic lesions in genes like *RET, MEN1, PRKAR1A* with an involvement in regulation of cell growth, differentiation and death.

Definitive treatment is timely screening and surgical resection of tumors. Information on mutation status facilitates effective prophylactic interventions obligatory for management and survival of the patients and their relatives.

The present study reports on mutation screening of *MEN1*, *RET* and *PRKAR1A* in 113 patients and 100 controls. Majority of the patients had characteristic clinical presentations.Some exhibited rare symptoms of acromegaly and infertility as in Carney complex, and another patient showed cutaneous lichen amyloidosis associated with MEN2A. Molecular analyses revealed *RET* hotspot mutation 'C634R' in 36% cases (Table-1). Novel and reported mutations and polymorphisms were identified in the genes screened. *In silico* analysis using computational algorithms predicted the changes to be pathogenic providing evidence for their role in disease causation. Predictive genetic testing was provided to 'at risk' family members to identify changes followed by extensive clinical evaluation of neoplastic changes if any. Translation of genetic findings was achieved by comprehensive evaluation followed by cost effective surveillance and prophylactic surgeries of asymptomatic family members wherever required.

Table-1 Mutations identified in the present study

S.No	Gene	Phenotype	Genotype	Position	Nature of mutation/SNP
1	*MEN1*	Hyperparathyroidism and gastrinoma	14 bp deletion	Exon8	**Novel**
2		Insulinoma	c.445-44G→A	Intron2	**Novel**
3		Insulinoma	M561K	Exon10	**Novel**
4		Insulinoma	c.913-42G→C	Intron6	**Novel**
5	*RET*	Medullary thyroid carcinoma and cutaneous lichen amyloidosis	C634R	Exon11	Reported
6	*PRKAR1A*	Cardiac myxoma, acromegaly and infertility	22bp insertion	Exon6	**Novel**

PS03.31

Clinical exome sequencing for improved diagnostics and treatment of patients visiting a multidisciplinary nephrogenetic outpatient clinic

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Single gene disorders are estimated to account for ~30% of children and ~10% of adult patients attending renal outpatient services. In the Radboudumc a multidisciplinary nephrogenetic outpatient clinic for children and adult patients with (genetic) kidney diseases has been established by a team of (pediatric) nephrologists and a clinical geneticist. Clinical exome sequencing for a broad spectrum of isolated- and syndromic renal (ciliary) disorders has been developed. The approach consists of a two-tier analysis in which the first step is to screen for pathogenic variants in genes that are known to be mutated in renal diseases (170 genes) or (renal) ciliopathies (125 genes). If causative mutations are not identified in the first step, the complete exome data set can be analysed with informed consent. The first results with the renal disease gene panel in 63 unrelated patients with undiagnosed renal disease led to pathogenic mutations in nine cases (14%), and in 11 other cases (17%) likely pathogenic variants needed follow-up studies. Further analysis of the complete exome data set in 17 patients, revealed novel candidate genes in two cases that are under investigation. In addition, copy number variation analysis of exome data revealed a pathogenic deletion of the *NPHP1* gene in two cases, confirming the clinical diagnosis. We conclude that the combination of the multidisciplinary outpatient clinic with diagnostic exome sequencing provides a powerful tool for detecting causative mutations in up to 23 out of 63 patients (35%) with a renal disease.

PM03.32

KOUNCIL: kidney-oriented understanding of correcting ciliopathies

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Introduction Nephronophthisis is an autosomal recessive renal ciliopathy that constitutes the leading monogenic cause of end-stage renal disease in children. The KOUNCIL consortium is a collaboration between the UMC Utrecht, the Radboudumc Nijmegen and UC London aimed at elucidating the genetic etiology and pathophysiological mechanisms underlying nephronophthisis and identifying drugs that prevent renal failure. Our goal is to improve early genome diagnostics, genetic counseling and therapeutic options for nephronophthisis patients.**Methods** We employ next-generation sequencing to identify novel disease genes in 100 nephronophthisis patients included within the AGORA biobank project. The functional effect of novel mutations is assessed using *in vitro* and *in vivo* models. Genotypic and phenotypic patient characteristics are registered in a nephronophthisis database, facilitating correlation analyses and the identification of early phenotypic markers. Newly identified genes are incorporated into a diagnostic targeted next-generation sequencing panel of ciliary genes. We use a systems-biology approach to identify and functionally characterize nephronophthisis-associated protein modules. Finally, we use high-throughput repurposing screens in zebrafish embryos to discover drugs that halt progression of renal failure.**Results** We expect to uncover the causal mutation in 60-90% of nephronophthisis patients. KOUNCIL members were involved in the identification of three novel genes (*IFT172*, *WDR34* and *WDR60*) for nephronophthisis-related disorders. Clinical guidelines and new diagnostic tools for nephronophthisis are developed and implemented in genome diagnostics. We expect to identify FDA-approved drugs that can lead to novel therapies for nephronophthisis.**Conclusion** The KOUNCIL study is designed to advance understanding of renal ciliopathies and improve clinical care for nephronophthisis patients. KOUNCIL is funded by the Dutch Kidney Foundation (CP11.18).

PS03.33

Diagnostics of autosomal recessive polycystic kidney disease by NGS

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Introduction: Mutations in the *PKHD1* gene cause autosomal recessive polycystic kidney disease (ARPKD). The *PKHD1* gene is located on chromosome 6 and consists of 68 exons. The incidence of ARPKD is approximately 1:30 000. Some newborns with ARPKD die due to insufficiently developed lungs. In those who survive, the disease is manifested by backbone and limb deformations, unusual facial features and distended abdomen. *Kidney function* deteriorates progressively towards renal failure. Fortunately, dialysis and transplantation can extend patients' lifespan.

Materials and Methods: The analysis of the *PKHD1* gene was carried out by next generation sequencing (GS-Junior, Roche). In-house designed primers were used for amplicon preparation. Amplicons cover all coding exons and exon-intron boundaries. Universal design for library preparation was used.

Results: DNA samples of eleven unrelated patients with symptoms of ARPKD were analysed. Thr36Met - a heterozygous mutation causal for ARPKD was identified in five samples. This mutation was accompanied by the second heterozygous mutation in all five cases. Those five mutations have been previously described in literature in patients with ARPKD, namely Arg92Trp, Gly112Arg, Leu2128Ter, Ile2331Lys and Ile2957Thr. In addition, heterozygous mutations - one considered as deleterious Gly1712Arg and one novel Gln1122Ser - were found in one sample. In five samples we did not find any deleterious or suspicious mutations.

Conclusion: Our method allows reliable analysis of the *PKHD1* gene to be achieved in a few days. To assess the clinical significance of novel mutations, it is necessary to analyze the *PKHD1* gene in the individual members of patients' families.

PM03.34

Evidence for a dosage-sensitive mutational network in a cohort of 308 patients with early and severe forms of polycystic kidney disease

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Polycystic kidney disease (PKD) is the most common potentially life-th-

reatening human genetic disorder. In addition to recessive ARPKD, 2-5% of patients with dominant ADPKD show early and severe disease. Genetic testing is cumbersome because of the size and structure of major disease genes and increasing genetic heterogeneity. We established a novel customized sequence capture based NGS panel for PKD that currently targets 95 genes. In total, we analysed a cohort of 308 patients with early and severe PKD. The majority of patients carried mutations in *PKD1, PKD2,* and *PKHD1*, however a subgroup harboured mutations in genes typically related to other ciliopathies such as nephronophthisis, Joubert, Meckel, and Bardet-Biedl syndrome. We demonstrate that *PKD1* is a driver for early and severe manifestations in families with dominant ADPKD. Notably, mutations in both ADPKD genes can also be identified in patients with recessive PKD. A proportion of patients carry aggravating mutations in more than just one single gene/allele in the context of a functionally proven dosage-sensitive network. Zebrafish and Xenopus are used as models for validation of some of our findings. This is the most comprehensive study performed so far by which we propose a dosage-sensitive model for early and severe forms of PKD. Our NGS panel allows the parallel analysis of all disease genes including the pseudogene-variable *PKD1* gene which plays a decisive role in cyst initiation. An accurate genetic diagnosis is crucial for genetic counselling, prenatal diagnostics and the clinical management of patients.

PS03.35

AN ATYPICAL AND RARE FAMILIAL PULMONARY FIBROSIS CASE WITH A PATHOGENIC SFTPC GENE MUTATION: DIAGNOSIS IN THE DETAIL

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Familial pulmonary fibrosis (FPF) is defined as idiopathic interstitial pneumonia (IIP) in ≥ 2 first-degree relatives with diagnosis based on established clinical criteria. The clinical findings of IIP are bibasal reticular abnormalities, ground glass opacities, or diffuse nodular lesions on high-resolution computed tomography and abnormal pulmonary function tests that demonstrate intrapulmonary restriction. Mutations in TERT, TERC, and SFTPC genes have been identified in about 8%-15% of individuals with FPF and 1%-3% of simplex cases with SFTPC mutations associated with Pulmonary surfactant metabolism dysfunction-2 (SMDP2), inherited in a autosomal dominant manner detected in ≤1% for FPF and simplex cases.
The 26 year old female proband presented after an immigration chest xray demonstrated increased bronchial wall markings in lower zones having had a 2 year history of shortness of breath. Subsequent investigations including HRCT chest and VATS lung biopsy showed appearances in keeping with extensive interstitial fibrosis with a usual interstitial pneumonia type picture. The only deceased sister had reported history of failure to thrive and recurrent pneumothoraces from 17. Subsequent genetic testing detected the proband to be a heterozygous carrier of a pathogenic SFTPC mutation which was maternally inherited.
Further presentation of the familial clinical phenotype and genetic investigation will be presented which illustrates the variable intrafamilial clinical phenotype, adds to the knowledge on SMDP2 gene phenotypic spectrum, the complexity of the diagnostic pathway with rare disease and need for close multidisciplinary liaison, whilst highlighting the importance of clarification of family history and phenotyping.

PM03.36

Integrating genetic analysis with phenotypes of biliary atresia

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We aim to explore the role of rare copy number variants (CNVs) in non-syndromic BA. We revisited clinical records of 89 non-syndromic type III BA patients with median follow up of 17.20 years, which revealed that 41.57% BAs were affected with chronic extra-hepatic diseases, with high prevalence of autoimmune-allergic diseases (22.47%) and Glucose-6-phosphate dehydrogenase deficiency (14.29% of the males). After genotyping on the genome-wide Affymetrix5.0 array, we shortlisted 29 'BA-CNVs' found in BA patients but not in the general population, and collated 103 BA-associated genes from a gene-based genome-wide association analysis on common variants, for downstream analysis. In BA-CNVs we discovered three categories of genotype-phenotype correlations: i) two de novo BA-CNVs, perturbing genes/chromosome-segments known to BA, correlated with BA; ii) three BA-CNVs encompassing genes known to immunity defects, correlated with comorbidities of those immune disorders in 3 carriers; iii) importantly, genes affected by BA-CNVs (N=102; gene set-1) were enriched with immune genes, correlated with the high prevalence of immunity disorders in BA. Further, we proved significant connectivity between gene set-1 and genes tagged by common variants (N=103; gene set-2) (Empirical p=0.039). As multiple function modules were elucidated in topological analysis of the BA candidate gene network, a 'core' position of cellular signalling pathways, which affect inflammatory and immunity regulation pathways, was highlighted.
Conclusions: BA-CNVs underpin BA phenotypic complexity, and converge with those BA-associated common variants in a molecular network implicated in BA pathogenesis. Integrating clinical/epidemiological data and BA genetic findings is plausible using the BA ‚diseasome' approach.

PS03.37

Steroid resistant nephrotic syndrome (SRNS): NGS panel testing to direct therapy and intervention

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Steroid Resistant Nephrotic syndrome (SRNS) is a disorder of the glomerular filtration barrier. It is characterised by massive proteinuria, hypoalbuminaemia and oedema, and is managed by non-specific heavy immunosuppression. An invasive biopsy is often required and the majority of patients progress to end stage renal failure. Rapid genetic diagnosis is important for therapy and intervention as genetic SRNS is non-responsive to immunosuppression, and has a lower rate of post transplant recurrence. SRNS is associated with over thirty genes expressed at the glomerular filtration barrier.
BGL has received >200 worldwide diagnostic referrals (80% paediatric and 20% adult) for NGS clinical panel testing of 37 SRNS genes (Haloplex; MiSeq; open-source pipeline, including CNV analysis using CONTRA). Likely pathogenic variants have been identified in 27% of referrals, most commonly occurring in NPHS1, NPHS2, WT1, COL4A3 and COL4A4 with a different mutation spectrum in adult and paediatric patients. A further 27% have candidate variants. Heterozygous CNV variants were detected in two patients with NPHS1 and NPHS2 related disease supporting CNV analysis in all patients
Variant stratification is challenging due to the presence of rare variants in under-sequenced populations. A pathogenic LMX1B mutation, c.737G>A, was associated with nail-patella-like renal disease, and a patient with a c.287C>T MYH9 variant presented with thrombocytopaenia and SRNS associated with MYH9-related disease. Several patients have variants in more than one collagen gene. Prenatal diagnosis was performed for a Pierson syndrome family with pathogenic LAMB2 variants, c.[4198_4199delCT];[928T>C].
We present our results illustrated by cases highlighting the clinical benefit of panel testing.

PM03.38

Role of CD2AP mutations in Steroid Resistant Nephrotic Syndrome revisited - new insights from next generation sequencing

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To date, mutations in over 50 genes have been published as underlying different cases of Steroid Resistant Nephrotic Syndrome (SRNS). SRNS is a molecularly heterogeneous condition but, in all cases, the underlying insult results in malfunction of the kidney's Glomerular Filtration Barrier (GFB) resulting in protein loss into the urine. CD2AP was first proposed as a site of dominant mutations underlying SRNS in 2003. This was supported by histological features of renal disease in heterozygous loss of function mouse mutants and a heterozygous splice site variant observed in two renal disease patients. Since then, further disease relevant alleles have been reported and the screening of CD2AP has been added to clinical protocols.
The advent of next generation sequencing technologies and the discovery of other causal genes questions whether mutations in CD2AP alone are sufficient to cause SRNS. Here, we present data on CD2AP variants found from whole exome sequencing of over 200 SRNS patients and 1500 controls. We show that several alleles previously described as disease causing are observed in our control population. In addition, whilst we observe rare alleles in CD2AP in two of our cases, in each of these individuals, we observe other rare alleles in other SRNS genes that are predicted to be functionally relevant. These observations demonstrate the challenges in determining the pathogenicity of individual variants in the absence of complete data. Our observations also highlight the potential oligogenic nature of certain forms of SRNS.

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PS03.39

A study to follow up patients after transient neonatal diabetes mellitus - an International Patient Register

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Transient Neonatal Diabetes Mellitus (TNDM 1), caused by overexpression of imprinted genes at 6q24 is characterised by growth restriction and diabetes that presents soon after birth and undergoes spontaneous remission in the first year of life. Individuals have a higher-than-normal risk of developing type-2 diabetes in later life, however the degree of risk is not known. The register includes over 180 cases of 6q24 TNDM and 72 of these families have given consent for follow-up and we have information on 17 individuals over the age of 5 years.
Results: 6/17 (35%) had experienced a recurrence of diabetes between 5 and 14 years (mean age 12y 8m). Permanent recurrence was reported in 4 cases which were treated with insulin. One recurrence was transient and concomitant with an episode of gastric flu at 5y. The remaining case was managed with metformin and diet at the follow up.
11/17 (65%) experienced no recurrence of diabetes at the time of follow up. Ages at follow up ranged between 5 years and 13 years 6 months (mean age at follow-up = 8y 2m)
In total, 7/17 (41%) were reported as having learning difficulties. Of these, 1 attended special school and had a diagnosis of autism and 1 suffered brain damage at birth and requires help with mobility and fine motor skills. The remaining 5/17 (29%) reported some developmental delay, notably in speech and language.
A survey is underway to expand these findings and gather further follow-up data. Additional results will be presented at ESHG.
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PM03.40

Urinary system and renal malformations in girls with Turner syndrome

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Turner Syndrome (TS), in which there is a loss of all or part of one X chromosome, occurs in 1 in 2500 to 1 in 3000 born females and is associated with characteristic findings. Renal and urinary system malformations with their posterior complications such as urinary tract infections or proteinuria have been recognized to increase in patients with TS. In this retrospective study we report a detailed clinical history and analyzed renal and urinary system pathology in 34 girls with TS. All TS patents were evaluated by renal and collecting system ultrasonography and if structural renal or urinary system malformations were found, cystourethrography and centellography (DMSA or DTPA) was used.
Patients mean age at renal and urological studies was 9,7 years (2-18 years). The cytogenetic findings in 34 patients with TS were classic in 20 patients (58,82%), mosaic and structural aberration of X chromosome: in 14 patients (41,18%). The prevalence of renal and urinary system pathology was 44,11% (15 patients). The most frequent findings were urinary system malformations 20,58% (7 patients), associated with renal malformations 8,82% (3 patients), while 5 patients (14,7%) had renal malformations alone. Horseshoe kidney, malrotation or other position abnormalities, duplication of the collecting system and different ureterovesicular obstruction were found.
Conclusion: The early diagnosis of renal and urinary system malformation in TS and their follow- up is crucial to reduce the morbility in these patients. There appears to be no correlation between karyotype and the presence or type of renal or urinary system malformation

PS03.41

Whole exome sequencing identifies two pathogenic variants in *NPHS2* and confirms the diagnosis of nephrotic syndrome type 2 in two siblings with atypical Alport syndrome

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The authors report a case of two affected sisters, born to non-consanguineous healthy parents, who were diagnosed with Alport syndrome, based on a family history of microscopic hematuria, steroid-resistant proteinuria, end-stage renal disease and sensorineural hearing loss.
Ultrastructural examination of the renal biopsy showed glomerular basement membrane (GBM) thinning and thickening with atypical electron-dense bodies. Detachment of GBM from podocytes was compatible with focal segmental glomerulosclerosis (FSGS). Pathogenic mutations in the *COL4A5*, *COL4A4* and *COL4A3* genes were excluded by direct sequencing of all exons and by MLPA of *COL4A5*. Subsequently, whole exome sequencing was performed in the proband, using the Illumina HiSeq2000 platform and filtering the variants on genes encoding podocyte products. Two substitutions, c.686G>A (p.(Arg229Gln)) and c.928G>A (p.(Glu310Lys)), were detected in the *NPHS2* gene, in compound heterozygosity in the affected sisters, confirming the diagnosis of autosomal recessive nephrotic syndrome type 2 and supporting genetic counselling in this family. FSGS is a glomerular lesion that results from podocyte detachment or death, clinically presenting with proteinuria and progressing to renal failure. It can have multiple primary or secondary causes, including monogenic disorders. Although typical ultrastructural GBM changes are seen in Alport syndrome (as alternating thinning and thikkening, lamellation and the presence of electron-dense bodies), FSGS may be observed in later stages of the disease. This work highlights the relevance of next-generation sequence in determining the molecular genetics diagnosis in suspected cases of Alport syndrome without *COL4A3*, *COL4A4* or *COL4A5* pathogenic mutations.

PS04.01

Mutations in PDE4D and molecular pathology of acrodysostosis without hormone resistance

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Acrodysostosis without hormone resistance is a rare skeletal disorder characterized by
brachydactyly, nasal hypoplasia, mental retardation and occasionally developmental delay. Mutations in the gene encoding cAMP-hydrolyzing phosphodiesterase-4D (PDE4D) have been reported to cause this rare condition but the pathomechanism has not been fully elucidated.
To understand the pathogenetic mechanism of PDE4D mutations, we conducted 3D modeling studies to predict changes in the binding efficacy of cAMP to the catalytic pocket in PDE4D mutants. Our results indicated diminished enzyme activity in the two mutants. Ectopic expression of PDE4D mutants in HEK293 cells demonstrated this reduction in activity, which was identified by increased cAMP levels. However, cells from an acrodysostosis patient showed low cAMP accumulation, which resulted in a decrease in the phosphorylated cAMP Response Element-Binding Protein (pCREB)/CREB ratio. The reason for this discrepancy was due to a compensatory increase in expression levels of PDE4A and PDE4B isoforms, which accounted for the paradoxical decrease in cAMP levels in the patient cells expressing mutant isoforms with lowered PDE4D activity. We propose that specific inhibitory PDE4D mutations can lead to the molecular pathology of acrodysostosis without hormone resistance but that the pathological phenotype may be dependent on an over-compensatory induction of other PDE4 isoforms that can be expected to be targeted to different signaling complexes and exert distinct effects on compartmentalised cAMP signaling.

PM04.02

Investigation of angiogenesis-associated genes with risk of acute musculoskeletal injuries in two South African populations

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Introduction: Genetic factors have been implicated with risk of anterior

cruciate ligament ruptures and Achilles tendon ruptures. The angiogenesis signalling pathway plays a key role in extracellular matrix remodelling following mechanical loading. Increased levels of angiogenic cytokines have been reported in injured tendons and ligaments.
Aim: To investigate if angiogenesis genes are associated with risk of acute musculoskeletal injuries in two South African populations.
Methods: *Anterior cruciate ligament rupture group*: 227 controls (CON) and 227 participants with ACL ruptures (ACL), of which 126 had non-contact mechanism of injury (NON). *Achilles tendon rupture group*: 125 controls (CON) and 45 participants with ruptures of the Achilles tendon (RUP). All participants were genotyped for the functional *VEGFA* rs699947, *VEGFA* rs1570360, *VEGFA* rs2010963, *KDR* rs2071559 and *KDR* rs1870377 polymorphisms. Haplotypes were also inferred for *VEGFA* and *KDR*.
Results: In the ACL study, the *VEGFA* rs699947 CC genotype (p=0.010, OR: 1.92, 95% CI: 1.17-3.17) was significantly over-represented within participants with non-contact ACL ruptures. The *VEGFA* rs1570360 GA genotype was significantly over-represented (p=0.007, OR: 1.70, 95% CI: 1.16-2.50) in the CON group (48%) compared to the ACL group (35%). Inferred haplotype analyses also implicated genomic regions spanning *VEGFA* and *KDR*. No independent significant differences were observed in the genotype frequency distributions between the Achilles tendon CON and RUP groups.
Conclusion: These novel findings provide preliminary evidence highlighting the potential biological significance of the angiogenesis signalling pathway in the aetiology of acute musculoskeletal injuries.
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PS04.03
Genetic Investigation of Anencephaly in Old Order Amish

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Neural tube defects (NTDs) are the second most common type of birth defect after congenital heart defects, affecting over 300,000 births globally each year. They encompass a group of complex congenital malformations of the central nervous system characterised by failure of the neural tube close correctly resulting in an opening in the spinal cord or brain during early embryonic development. Anencephaly is a severe form of NTD, resulting in the absence of a major portion of the brain, skull, and scalp and is a lethal defect. We investigated an extensive eight-generation pedigree from the Amish community with 4 interlinking families with anencephalic births indicative of an autosomal recessively-acting mutation. Assuming that a founder mutation was responsible, we used genome-wide SNP mapping to identify regions of autozygosity in a single affected case likely to harbour the disease gene. This identified three candidate regions located on chromosomes 14q, 3p and 1p. This data was then cross-referenced with exome sequencing data of the affected case to identify the putative causative mutation.
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PM04.04
Association of IL1B and IL6 gene polymorphisms with higher disease activity and clinical pattern of psoriasic arthritis.

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Objective: Psoriatic Arthritis (PsA) is a chronic inflammatory disease associated to psoriasis that affects peripheral joints, the spine and enthesis. As result of the therapeutic success of antagonists of TNF and the IL-23/IL-17 cytokines axis, we know these cytokines play an important role in its pathogenesis. IL-23 is necessary for the differentiation and survival of IL-17 producing helper lymphocytes (Th17) IL-1B and IL-6 also is needed for the differentiation of Th17 cells. Here, we have analyzed whether IL1B (rs16944) and IL6 (rs1800795) polymorphisms are associates with inflammatory activity, radiographic damage or clinical pattern of Psoriatic Arthritis (PsA).
Patients and Methods: 125 patients suffering from PsA were included in the study. Genomic DNA was extracted from peripheral blood using phenol/chloroform procedure and genotyped using TaqMan 5'-exonuclease allelic discrimination assays (Applied Biosystems). Statistical analysis was performed using SPSS software.
Results: The G allele of IL1B rs16944 was associated with higher peripheral joint disease activity (OR: 3,13; P<0,0004; CI95%: 1,43-6,82 p<0,008 (corrected), while the G allele of the IL6 rs1800795 presented a strong trend to be associated with peripheral forms (70,86%) (OR: 1,89; p<0,03; CI95% 1,06-3,39, p: 0,05 (corrected). In addition, this allele showed a lower association with HLA-B27 (15,78%) compared with C allele (28,57%) (OR: 0,469; p=0,02; CI95% 0,238-0,923, corrected P: 0,03). None of the polymorphisms were associated to radiological damage.
Conclusions: IL1B rs16944 and IL6 rs1800795 could modulate the expression of IL-1[[Unsupported Character - Symbol Font ]] and IL-6, having clinical implications in PsA.
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PS04.05
Molecular characterization and transcriptome-wide expression profiling of two patients affected with spondyloepimetaphyseal dysplasia with joint laxity type 1

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Mutations in *B3GALT6*, encoding the galactosyltransferase II (GalT-II) involved in the synthesis of the glycosaminoglycan (GAG) linkage region of proteoglycans (PGs), have recently been associated with a spectrum of connective tissue disorders, including spondyloepimetaphyseal dysplasia with joint laxity type 1 (SEMDJL1) and Ehlers-Danlos-like syndrome. Here, we report on two sisters compound heterozygous for two novel *B3GALT6* mutations that presented with severe short stature and progressive kyphoscoliosis, joint hypermobility, hyperextensible skin, platyspondyly, short ilia, and elbow malalignment. Microarray-based transcriptome analysis revealed the differential expression of several genes encoding extracellular matrix (ECM) structural components, including *COMP*, *SPP1*, *COL5A1*, and *COL15A1*, enzymes involved in GAG synthesis and in ECM remodeling, such as *CSGALNACT1*, *CHPF*, *LOXL3*, and *STEAP4*, signaling transduction molecules of the TGFβ/BMP pathway, i.e., *GDF6*, *GDF15*, and *BMPER*, and transcription factors of the *HOX* and *LIM* families implicated in skeletal and limb development. Immunofluorescence analyses confirmed the down-regulated expression of some of these genes, in particular of the cartilage oligomeric matrix protein and osteopontin, encoded by *COMP* and *SPP1*, respectively, and showed the predominant reduction and disassembly of the heparan sulfate specific GAGs, as well as of the PG perlecan and type III and V collagens. The key role of GalT-II in GAG synthesis and the crucial biological functions of PGs are consistent with the perturbation of many physiological functions that are critical for the correct architecture and homeostasis of various connective tissues, including skin, bone, cartilage, tendons, and ligaments, and generates the wide phenotypic spectrum of GalT-II-deficient patients.

PM04.06
Variants in the RANK gene are associated with bone mineral density and fracture risk in Maltese postmenopausal women

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Introduction: Osteoporosis is a progressive multifactorial skeletal disease characterised by low bone mass thereby increasing fracture susceptibility. The Receptor activator of nuclear factor Kappa B (RANK) is involved in the regulation of osteoclastogenesis via the RANK-RANK ligand-Osteoprotegerin pathway. The influence of two polymorphisms rs3018362 (A>G) and rs884205 (G>T) in the 3' untranslated region of this gene were analysed in relation to bone mineral density (BMD) and different low trauma fractures in Maltese postmenopausal women.
Materials and Methods: 1045 women were recruited and subdivided in three BMD control groups if without a fracture history: normal, osteopenic or osteoporotic. Cases were women who suffered any type of low trauma fracture. Genotyping of the rs3018362 polymorphism was performed by polymerase chain reaction and restriction enzyme digest, whereas real-time PCR was used for the rs884205 variant. Odds ratios were computed using logistic regression analysis adjusted for age and clinical risk factors.
Results: Homozygosity for the rs3018362 G allele was associated with a low femoral neck BMD (Adjusted OR=2.2 [95% confidence interval 1.1-5.1], p=0.02), and to a lower extent reduced lumbar spine BMD (OR=1.9 [1.1-3.4], p=0.04) relative to research subjects with a normal BMD. Cases carrying two copies of the minor allele T for the rs884205 variant had an increased fracture risk (OR=2.6 [1.1-7.1], p=0.04), especially that of the hip (OR=3.2 [1.2-8.0], p=0.02) and humerus (OR=2.9 [1.1-7.9], p=0.04). Haplotype-based analysis revealed that the alleles were not in linkage disequilibrium.
Conclusion: *RANK* gene polymorphisms predispose to reduced BMD or increased fracture susceptibility in Maltese postmenopausal women.

PS04.07

An unusual type of brachydactyly with delayed ossification and symphalangism

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We present the case of a now two year old boy. The second child of healthy nonconsanguineous Dutch parents. This boy has normal growth and development and is otherwise healthy. He presented at birth with abnormal positioning of both hands and feet, with abnormal implantation of fingers and toes. The fingers show absent flexion and extension creases over the proximal interphalangeal joints. There is no limitation of movement. Radiological examination at birth showed absent ossification of the preaxial metacarpals and metatarsals. In addition abnormal or absent ossification of the proximal and middle phalanges of the 3rd-5th fingers and absent ossification of the proximal and middle phalanges of the 2nd-5th toe was seen. Ultrasound investigation showed the presence of cartilage at the site of the metacarpals/tarsals and phalanges with the impression of symphalangism between the proximal and middle phalanges of the hands. At age 16m there is irregular ossification of the previously unossified metacarpals, metatarsals and phalanges with symphalangism of the proximal interphalangeal joints in the hands.
X-ray investigation of the spine, pelvis and long bones did not show additional abnormalities. DNA analysis of the NOG gene was normal and Cytoscan HD array analysis showed a normal male profile. We'll discuss overlap with previously described syndromes and suggest this is a new type of brachydactyly.

PM04.08

Report of two novel mutations in PTHLH as a cause of brachydactyly type E

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Introduction: Autosomal-dominant brachydactyly type E (BDE) is a congenital limb malformation characterized by small hands and feet as a result of shortened metacarpals and metatarsals. BDE can be isolated or associated with a syndrome, and in most cases, the genetic cause of BDE remains unknown. One of the genes associated to syndromic BDE is PTHLH, the gene coding for parathyroid hormone related protein (PTHrP), implicated in the regulation of the balance between chondrocyte proliferation and the onset of hypertrophic differentiation during endochondral bone development. Haploinsufficiency of PTHLH has been reported as the cause of BDE, mostly associated to short stature in 6 families.
Subjects and Methods: We report a family case with BDE, short stature and apparently breast hypoplasia in the daughter; and an isolated case, presenting with isolated BDE. PTHLH gene coding exons and coding exon intron-joints of all transcripts were analysed by direct sequencing.
Results: The sequencing of PTHLH gene revealed two intragenic deletions affecting the splicing (47_101+73del128 and c.101+3AAGT) as the cause of the syndrome. Both mutations are predicted to cause a mistake in the splicing, resulting in an aberrant mRNA, which will be degraded by nonsense-mediated decay and produce haploinsufficiency of the PTHrP in both patients.
Conclusion: This is the third report on PTHLH mutations leading to BDE revealing that the spectrum of symptoms is wider than previously described.
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PS04.09

The cell signalling pathway and risk of anterior cruciate ligament (ACL) ruptures.

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Polymorphisms within genes encoding signalling molecules have previously been implicated in risk of chronic Achilles tendinopathy. This study aimed to determine if these genetic variants are associated with ACL injury risk.
Methods: In total, 232 control (CON) and 234 ACL rupture participants (ACL), of which 135 had a non-contact mechanism of injury (NON) were genotyped for functional polymorphisms: IL1B rs16944, IL6 rs1800795, IL6R rs2228145, CASP8 rs3834129, CASP8 rs1045485, PTGER4 rs4495224, TGFB2 rs7550232, TNF rs1800629, TNF rs1799964 and TNFRSF1B rs1061622. Haplotypes were inferred for CASP8 and TNF. Statistical analyses were conducted ($p<0.05$) between the groups (CON vs. ACL and CON vs. NON). Sex-specific interactions were investigated.
Results: No significant differences were observed in the genotype and allele frequency distributions when all participants were analysed. The CASP8 rs3834129 del allele was significantly under-represented in the male CON group (41%) compared to the male NON subgroup (51%) ($p=0.047$, OR: 1.46, 95%CI: 1.01-2.12). The IL1B rs16944 TT genotype was significantly under-represented in the female CON group (10%) compared to the female NON subgroup (26%) ($p=0.029$, OR: 3.06, 95%CI: 1.09-8.64). The ins-G CASP8 haplotype was significantly over-represented in the CON group (55%) compared to the ACL (48%, $p=0.017$) and NON subgroup (48%, $p=0.031$). Similar results were observed in the male participants [ins-G: CON (56%) vs NON (43%), $p=0.040$].
Conclusions: This study implicated variants within CASP8 and IL1B with ACL ruptures, highlighting the biological significance of the cell signalling pathway in the etiology of ACL ruptures.
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PM04.10

Aquagenic palmoplantar keratoderma as a CFTR-related disorder: confirmation of a separate entity

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A 27 year-old caucasian female was referred to the dermatology clinics for swelling and discoloration of palms and soles a few minutes after bathing or swimming since one year, alongside with hypohidrosis. A single episode of pneumonia at 3 y was reported. On examination, *tinea manuum* and, a few minutes after water exposure, a rapid onset of a papillomatous medio-palmar swelling was observed. Her growth parameters were normal without any chest deformity. Her sinus X-rays indicates absence of nasal polyps. A diagnosis of aquagenic palmoplantar keratoderma (APK) was made. A sweat test indicated chloride concentrations of 64 mmol/l and 51 mmol/l (N<60). Since APK has been already reported in association with clinical cystic fibrosis and/or *CFTR* mutations, next generation sequencing of *CFTR* was undertaken and identified a combination of apparent homozygosity for a missense *CFTR-RD* mutation c.2855T>C (p.Met952Thr), already described in congenital bilateral absence of *vas deferens,* and a large heterozygous deletion encompassing *CFTR* exons 16 to 20, described as c.2620-674_3367+198del9855 known as CF pathogenic mutation. Based on these results, we confirm the existence of a link between isolated APK and *CFTR* mutations and suggest to include this phenotype (APK only) at the mild end of CFTR clinical spectrum. Owing to the small number of CFTR-related APK cases reported sofar, we are unable to conclude on the importance of *CFTR* mutation-negative APK. This observation also suggests that screening for rare *CFTR* mutations should be systematically proposed in case of isolated APK in order to provide appropriate genetic counseling.

PS04.11

FAM110B on 8q12.1 as a new candidate gene for CL±P in a high-prevalence area in South America

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Background: In South America a cleft lip with or without cleft palate (CL±P) high prevalence region were detected in Patagonia (Argentina). Amerindian ancestry was identified as a risk factor. We aimed the identification of autosomal genomic regions that may contribute to CL±P in this probably more homogeneous population.
Method: The study sample included 30 families with isolated CL±P (31 affected and 113 total individuals). They were genotyped on the Affymetrix Genome-Wide 6.0 array. We calculated linkage disequilibrium (LD) between each pair of SNPs into a window of 50 SNPs, shifting the window 5 SNPs forward and repeating the procedure to scan all autosomes. Then we pruned the data removing one SNP of each pair that was in strong LD (r >0.8). We performed a segmentation analysis to obtain genomic regions significantly associated with CL±P. We identified genomic segments of a maximum length of 250Kb with more than one SNP with p-value less than 0.001.
Results: A total of 14 genomic segments with two or more independent SNPs significantly associated with CL±P were identified. A segment of 61.3Kb on 8q12.1 containing FAM110B gene showed the most significant association with CL±P (p= 0.00007). Other segment of 25.5Kb on 3q29 significantly associated with CL±P (p=0.0002) was close to the FGF12 gene that was previously associated with oral cleft phenotype in other populations.
Conclusion: Our results suggest FAM110B on 8q12.1 as a new candidate gene to CL±P. The region on 3q29 near FGF12 gene should be more investigated because previous associations with CL±P.
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PM04.12
Report of a Saudi Family with Kniest dysplasia (KD) showing an Autosomal Recessive Inheritance

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Kniest Dysplasia (KD) is a rare autosomal dominant spondylo-epiphyseal dysplasia (OMIM # 156550) characterized by a disproportionate short stature, midfacial hypoplasia, progressive myopia, bell-shaped chest, kyphoscoliosis, stiffness and enlargement of the joints, premature osteoarhritis, and contractures of fingers.
Often, it is associated with cleft palate, Pierre-Robin sequence and deafness. KD is caused by a mutation in the COL2A1 gene encoding type II collagen. Here we describe two affected siblings born to a phenotypically normal Saudi Arab first cousins couple. They were born with prominent eyes, cleft soft palate, micrognathia, midfacial hypoplasia, knee joint contracture, and short lower limbs (birth length was 43 cm). Both developed severe myopia which progressed to left-sided retinal detachment in the younger affected sister at 4 years of age, hearing impairment, disproportionate short stature, barrel-shaped chest, thoracolumbar kyphoscoliosis, knee and hip joint flexion contractures, and bowed legs. Radiographic study showed dumbbell shaped femurs and humeri, hypoplastic pelvic bones, platyspondyly, flared metaphyses, and large epiphyses.
Molecular analysis revealed the presence of a "de novo" homozygous splice site mutation in the COL2A1 gene (c.2904+2T>C) in both of the affected siblings.
The parents were found to be heterozygous for the same mutation. Both had an appropriate height, and their radiographic study, hearing test and ophthalmological evaluation were normal). To our knowledge, this is the first reported family in whom two siblings with KD are found to have an identical biallelic splice site mutation in the COL2A1 gene.

PS04.13
Report on a patient with extremely fragile skin, dermatosparaxis, joint hypermobility, short stature, skeletal deformities, and lipomas: a new syndrome?

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We report on a patient who came to our attention with a suspicion of Ehlers-Danlos syndrome (EDS). The proposita, a 56-year-old Italian woman, was born in a geographically isolated valley from healthy and referred non-consanguineous parents, and had two healthy sons. Redundant, sagging, fragile skin with atrophic scars was present since infancy. In adulthood the cutaneous involvement was worsening leading to generalized dermatosparaxis. At examination, generalized photoageing and capillary fragility, deep facial wrinkles and palmar creases, blue sclerae, palpebral ptosis, short stature (150 cm, lower than the 3rd centile), brachydactyly with low-set thumbs, barrel chest, deforming osteoarthritis, bilateral hallux valgus, joint hypermobility according to Beighton score (7/9), varicose veins, and frontal alopecia with thin hair, were observed. Chronic pain, recurrent bursitis, several excisions for lipomas, multiple muscle ruptures, and spontaneously solved subarachnoid hemorrhage, were referred. Immunohistological examination of a skin biopsy revealed epidermal thinning with reduced keratinocytes' layer and increased and morphologically altered elastic fibers resembling solar elastosis, without calcium deposits, and reduced collagen fibers. Cutis laxa and the dermatosparaxis and classic EDS types were excluded for the peculiar clinical presentation of the patient, not yet described to our knowledge. Furthermore, mutational screening of the *ADAMTS2*, *COL5A1* and *COL5A2* genes did not reveal causal mutations. The plausible hypothesis of a recessive inheritance prompted us to perform SNP array analysis that showed the presence of several large chromosomal regions with loss of heterozygosity. We expect that in these candidate regions whole exome sequencing will reveal the causal mutation involved in this disorder.

PM04.14
Craniofrontonasal syndrome with central polydactyly: Case report

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Craniofrontonasal syndrome (CFNS), also known as craniofrontonasal dysplasia, is an X-linked developmental disorder, caused by mutations in the EFNB1 gene. EFNB1 located at Xq13.1, and encoding a ligand of the Ephrin family of receptor protein tyrosine kinases. This syndrome is characterized by craniofrontonasal bridge and bifid nasal tip, frontal bossing, coronal suture synostosis (unilateral or bilateral), hypertelorism, frizzy and curly hair, corpus callosum agenesis, and cleft lip or palate. Skeletal features include Sprengel shoulder, dysplastic clavicles, partial cutaneous syndactyly of the hands and feet, duplication of the thumbs or halluces and characteristic longitudinal ridging and splitting of nails. Herein we present a girl CFNS patient with a heterozygote c.196 C>T (p.R66*) mutation in EFNB1. The patient also had central polydactyly of the right hand which has not previously been described in this syndrome.

PS04.15
In vitro functional characterization of the Bardet Biedl syndrome-9 gene in nonsyndromic craniosynostosis.

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Introduction: The molecular mechanisms underlying nonsyndromic craniosynostosis (NSC) are still largely unknown. Recent evidence obtained thorugh GWAS on a large cohort of patients indicated the significant association of sagittal NSC to the Bardet Biedl Syndrome-associated gene 9 (BBS9). BBS9 is involved in the formation of the primary cilium. Preliminary data demonstrated that cells isolated from prematurely fused sutures of midline NSC patients display aberrant BBS9 expression and reduced number of primary cilia, affecting the cell osteogenic potential. This study was aimed at characterizing the BBS9 gene as associated to midline NSC.
Methods: Calvarial-derived mesenchymal cells (CMSC) were isolated from fused and patent sutures of NSC patients. The expression of BBS9 was analyzed through qPCR, both in fused and patent samples. The BBS9 gene was silenced by siRNA in fused-CMSC, the effect of gene modulation on ciliogenesis was analyzed by immunofluorescence.
Results: BBS9 level was higher in fused-CMSC compared to controls. Confocal microscopy showed that fused-CMSC display a predisposition to produce differently shaped and developed primary cilia. 48 hours of siRNA treatment efficiently reduced the BBS9 level and affected the ciliogenesis, restoring a phenotype like patent-CMSC.
Conclusions: These data confirmed the association between BBS9 expression and aberrant ciliogenesis, suggesting that the dysregulation of primary cilium and its related signaling could underlie the alterated osteogenic process occurring at the site of premature synostosis in NSC.

PM04.16

Non-coding variant in the BMP2 locus associated with sagittal non-syndromic craniosynostosis causes differential GFP expression in zebrafish.

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Craniosynostosis is a common congenital malformation in which one or more of the cranial sutures of an infant skull fuse prematurely. Sagittal non-syndromic craniosynostosis (sNCS) has an estimated prevalence of ~2 per 10,000 live births. Our recent GWAS study identified robust associations to locus on chromosome 20 that is 345kb 3' of *BMP2*. We did not identify any coding *BMP2* variant, suggesting that variants in the associated region near BMP2 may harbor regulatory elements responsible for the phenotype. To test this we monitored the expression of *BMP2* in primary calvarial osteoblasts from sNCS patients. We observed overexpression of *BMP2* and heightened BMP2-signaling in 2 out of 8 osteoblast cell lines. In order to determine if rs1884302 variation causes functional changes, a 716 bp fragment was cloned into a zebrafish enhancer detector (ZED) vector with the wild-type allele (T) or the risk allele (C). Zebrafish transgenesis carried out with both fragments resulted in a strong expression of green fluorescent protein (GFP) in the head of the transgenic fish with a C, but not with the T alllele of rs1884302. Our *in vitro* results suggest that overexpression of *BMP2* by altered regulatory activity near this gene contributes to the etiology of sNSC, while *in vivo* results indicate that the differences in regulatory activity are dependent on the C or the T allele.

PS04.17

Modeling Craniosynostosis in zebrafish: How to investigate genetic-linked human skull deformations in fish

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Craniosynostosis is a human disease condition, in which premature fusion of cranial sutures results in prominent skull deformations. Genetic triggers for these disorders are numerous and are mostly linked to mutations in well-described developmental genes, like *FGFR3* and *TWIST1*. To investigate the function of novel or so far ill-described genes causing craniosynostosis, a reliable animal model is needed, allowing *in vivo* investigations of skull development over time and genetic engineering. Besides mouse, the zebrafish (*Danio rerio*) has become a good choice to study such complex developmental processes in a vertebrate model system.

We performed initial investigations of normal cranial suture development in zebrafish with different techniques and found promising similarities to human cranial development. Especially non-invasive techniques open up the possibility to repeatedly investigate cranial structures over time in single individuals. Conservation of gene expression patterns of craniosynostosis-associated genes in zebrafish hint to the notion that a similar genetic network is established in both species to form sutures. To decipher this network in more detail, we used gene expression analyses and different transgenic reporter lines for visualization of cellular processes. To furthermore conduct functional studies, we introduced site specific genomic alterations in the homologue of *TCF12* via CRIPSR/Cas9. The zebrafish carrying deletions in *tcf12* develop cranial deformations resembling the patients´ phenotypes. Our findings demonstrate that zebrafish is a potent animal model for investigating craniosynostosis and that it is possible to elucidate the functions of causative human mutations in fish.

PS04.19

Whole-exome sequencing approach revealed Carvajal syndrome as a differential diagnosis for Acrodermatitis enteropathica

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Background: Acrodermatitis enteropathica (AE; MIM #201100) is a rare and severe autosomal recessive zinc deficiency disorder caused by a biallelic anomaly of zinc transporter gene SLC39A4. It is characterized chiefly by acral and periorificial dermatitis occurring either in the perinatal period, or at weaning in breast-fed children. Its clinical diagnosis has to be necessarily confirmed by a genetic testing, as it may be misdiagnosed as various skin diseases ranging from relatively common ones to rare ones. Our present study is based on an Iranian inbred family with four living members having clinical symptoms suggestive of AE, albeit exhibiting no mutations either in SLC39A4 or in nearly 50 genes directly involved in zinc homeostasis.

Purpose: Our goal was to determine the genetic cause of the AE-like genodermatosis observed in this multiplex family.

Method: Whole-exome sequencing was performed in nine of the family members, including three affected and six unaffected ones.

Results: An exceedingly rare variant was found in the promoter of DSP (desmoplakin gene), which creates an alternative initiation codon. The deleterious effect of this variant was assessed by immunohistochemical and functional studies using luciferase-based assays. This result allowed us to diagnose Carvajal syndrome (MIM #605676), that is a rare autosomal recessive form of palmoplantar keratoderma associated with wooly hair, and with dilated cardiomyopathy, which last symptom was not observed in our patients.

Conclusions: This case not only illustrates the difficulty to recognize AE basing on clinical symptoms and pathology only, but it also emphasizes the usefulness of whole-exome sequencing in its differential diagnosis from other syndromes with overlapping features.

PM04.20

Pure hair and nail ectodermal dysplasia in a consanguineous Turkish family with a novel mutation in HOXC13 gene

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INTRODUCTION: Pure hair and nail ectodermal dysplasia (PHNED) is a rare disorder which characterized by hypotrichosis/ complete alopecia and nail dystrophy. Recent studies reported recessive families with PHNED having mutations in KRT85 and HOXC13 genes.

MATERIALS AND METHODS: We performed whole exome sequencing (WES) in a patient of PHNED phenotype from a consanguineous family. WES analysis revealed a novel homozygous frameshift mutation (c.353delC; p.T118fs) in HOXC13 gene.

CONCLUSION: To best of our knowledge this is the first Turkish family reported PHNED with novel HOXC13 mutation. In this report we will discuss this novel mutation with the review of the literature.

PS04.21

Refining palmoplantar keratodermal-congenital alopecia syndrome, Wallis type in two unrelated Italian patients

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In 2010, a classification of palmoplantar keratoderma-congenital alopecia syndrome (PPKCA) was proposed with two distinct phenotypes: a benign form with occasional dominant transmission (Stevanovic type; ten patients from four families) and a more severe variant with pseudoainhum, sclerodactyly and recurrence in sibs (Wallis type - PPKCA-WT; seven patients from two families). In the same report, a 10-year-old, sporadic Italian girl was reported as a further case of PPKCA-WT. Here, we describe PPKCA-WT in an additional 18-year-old Italian woman with widespread keratosis pilaris, ulerythema ophryogenes, marked hypotrichosis, and palmoplantar keratoderma progressively causing contractures, pseudoainhum and sclerodactyly. Due to the non-congenital nature of palmoplantar involvement, this patient was previously considered affected by IFAP syndrome. We also present the 5-year follow-up of the patient published in 2010, who demonstrates progression of the disease with worsening of sclerodactyly and pseudoainhum. Sanger sequencing of *GJB2*, *GJB6*, *LOR* and *MBTPS2*, as well as array-CGH (mean resolution: 200 Kb) excluded any pathogenic change in at least one subject. These patients represent two unrelated examples of an extremely rare genodermatosis clinically distinguishable from partially overlapping conditions, including Clouston, Lelis Olmested, KID and HOPP syndromes,

KFSD/IFAP syndrome, and odonto-onycho-dermal dysplasia. Although literature indicates autosomal recessive as the most likely inheritance, we cannot exclude a dominant or X-linked *de novo* mutation in these patients. A next-generation sequencing project is ongoing aimed at identifying the causative gene(s).

PM04.22
Mutational spectrum of hypohidrotic ectodermal dysplasia in Mexican mestizo patients

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Hypohidrotic ectodermal dysplasia (HED) is characterized by hypotrichosis, oligo/anodontia and hypohidrosis. HED generally has an X-linked pattern of inheritance, autosomic dominant and recessive cases have been described. The affected genes are *EDA* (Xq12-q13.1); *EDAR* (2q11); *EDARADD* (1q42.2-q43) and *WNT10A* (2q35). Objective: to describe a cohort of Mexican mestizo patients with HED, their mutational analysis and phenotype-genotype correlation. Material and methods: fifteen patients with HED belonging to fourteen different families were included. MLPA for the four genes was performed for dosage alteration in all patients; *EDA* sequencing was carried out in nine patients. Results: Index cases were males (6 months to 24 years of age), eight of them showed an X-linked pattern of inheritance. The following mutations were characterized: Case 1: deletion of exon 1. Cases 2 and 3, two affected brothers (case 3 also suffers from Down syndrome): novel mutation c.1037G>C, p.C346W in exon 8. Case 4: previously reported variant c.467G>A; p.R156H in exon 2. Case 5: the described mutation c.1311C>T; p.R357W in exon 8. Case 6 presented the variant c.466C>T, p.R156C in exon 2. Cases 7, 8 and 9 exhibited a recurrent mutation c.463C>T, p.R155C in exon 2. Maternal samples of cases 2, 3 and 6 were unavailable for molecular study but the clinical data suggested a heterozygous genotype. Carrier state was molecularly confirmed on the remaining mothers; all but one had clinical data. Conclusion: our results confirmed the low frequency of related genes dosage alterations and the high prevalence of X linked HED in Mexican population.

PS04.23
Genetic heterogeneity and clinical variability in musculocontractural Ehlers-Danlos syndrome caused by impaired dermatan sulfate biosynthesis

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Bi-allelic mutations in *CHST14*, encoding dermatan-4-*O*-sulfotransferase-1 (D4ST1), cause musculocontractural EDS (MC-EDS), a recessive disorder characterized by connective tissue fragility, craniofacial abnormalities, congenital contractures and developmental anomalies. More recently, a bi-allelic mutation was also identified in the *DSE* gene, encoding dermatan sulfate epimerase-1 (DS-epi1), in a child with MC-EDS features, thereby suggesting locus heterogeneity for this condition. DS-epi1 and D4ST1 are crucial for biosynthesis of dermatan sulfate (DS) moieties in the hybrid chondroitin sulfate (CS)/DS glycosaminoglycan (GAG) chains of proteoglycans. We report four novel families with severe MC-EDS caused by unique homozygous *CHST14* mutations and the second family with a homozygous *DSE* missense mutation, presenting a somewhat milder MC-EDS phenotype. The glycanation of the dermal DS proteoglycan decorin is impaired in fibroblasts from D4ST1- as well as DS-epi1-deficient patients. However, in D4ST1-deficieny the decorin GAG is completely replaced by CS, whereas in DS-epi1-deficiency still some DS moieties are present. The multisystemic abnormalities observed in our patients support a tight spatiotemporal control of the balance between CS and DS, which is crucial for multiple processes including cell differentiation, organ development, cell migration, coagulation and connective tissue integrity.

PM04.24
The Ehlers-Danlos syndrome type VI spectrum: a genetically heterogeneous group of clinically overlapping conditions

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The Ehlers-Danlos syndrome kyphoscoliosis type (EDS VIA) is an autosomal recessive disorder characterized by severe kyphoscoliosis and neonatal muscular hypotonia, in addition to the classical signs of EDS, such as joint hypermobility and a hyperextensible and fragile skin. EDS VIA was the first inborn error of collagen metabolism to be solved at the biochemical and molecular level. It is caused by mutations in *PLOD1* leading to deficient activity of lysylhydoxylase 1 (LH1), a collagen-modifying enzyme. Patients with a phenotype resembling EDS VI but with normal LH1 activity were originally classified as EDS VIB. Several studies have now shown that EDS VIB comprises a genetically heterogeneous spectrum that includes defects in dermatan sulfate biosynthesis (musculocontractural EDS, MC-EDS) and defects in the transcriptional regulation of collagen and other extracellular matrix genes (brittle cornea syndrome, BCS). This study reports on the natural history of 31 EDS VI patients (EDS VIA n=12; MC-EDS n=10; BCS n=9). In addition, we critically reviewed the clinical features of 135 molecularly proven EDS VI patients (EDS VIA n=68; BCS n=48; MC-EDS n=19). We provide a comprehensive overview of the clinical characteristics of these three disorders and highlight the disorder-specific features. Our results show that despite the unmistakable clinical overlap, EDS VIA, MC-EDS and BCS are three distinct disorders. Based on this we propose to refine the outdated criteria for EDS VI, which should facilitate early and accurate clinical diagnosis of these rare, but often severe, syndromes.

PS04.25
Two cases of patients with Ehlers-Danlos Syndrome Type VIII and hoarseness

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Ehlers-Danlos syndrome (EDS) encompasses a genetically and clinically heterogeneous group of connective tissue disorders, characterised by joint hypermobility, skin hyperextensibility and tissue fragility. Type VIII EDS is very rare. Clinical features include severe, early-onset periodontitis, skin fragility and abnormal scarring. We report two cases of patients with EDS type VIII and hoarseness. The first patient is a 24 year old male from Bangladesh, the only child of unrelated parents. No other family members are affected. He has marked skin fragility, with easy bruising and splitting on his shins and elbows, early onset periodontitis and chronic hoarseness, the latter due to bilateral vocal cord sulci. The second patient is a 33 year old white Caucasian woman with premature loss of her primary teeth and adult teeth, pretibial bruising and a hoarse voice since her twenties. Her father also had early onset periodontitis. She has two-year-old twin boys who are also likely to be affected. She had an abnormality of her cricoarytenoid and underwent surgical correction. MRI scan revealed subglottic stenosis. While EDS VIII is clinically indistinguishable from vascular EDS, both patients had normal collagen protein analysis and COL3A1 sequencing, ruling out vascular EDS. Voice abnormalities have been described with other EDS subtypes and may result from defects in the collagen of the vocal ligament. Twenty-seven percent of patients with EDS subtypes I, II, III, IV and VI have self-reported dysphonia. Our cases demonstrate rarely reported laryngeal abnormalities and dysphonia, which is not a recognised feature of EDS VIII.

PM04.26
Molecular genetic diagnostics of epidermolysis bullosa in the Czech Republic

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Introduction:
Epidermolysis bullosa (EB) is a clinically and genetically heterogeneous group of disorders. There are four major types of inherited EB: EB simplex,

junctional EB, dystrophic EB, and Kindler syndrome. A characteristic feature of all EB types and subtypes is the presence of skin blistering and erosions.
Methods: EB families presenting at our laboratory over a period of 10 years were assessed using PCR-direct sequencing (KRT5, KRT14, TGM5, COL7A1) and/or sequence capture and targeted resequencing (COL7A1, KRT5, KRT14, DSP, DST, JUP, PKP1, EXPH5, TGM5, PLEC, COL17A1, LAMA3, LAMB3, LAMC2, ITGA6, ITGB4, ITGA3, FERMT1).
Results: Mutations associated with EB were identified in 139 probands: 22 and 42 patients had dominant and recessive mutations in COL7A1, respectively; 16 patients in KRT14; 10 patients in KRT5; 1 patient in PLEC; 36 patients in TGM5; and 1 patient in COL17A1. Eleven patients are without causal mutations despite that all known genes up to now associated with EB were analysed. Totally, mutations were identified in 128 probands (92%).
Conclusion: Besides identification of the spectra of mutations in Czech EB patients, the study present new method implemented into genetic diagnostics of genodermatoses in the Czech Republic - sequence capture and targeted resequencing - that provides more complete diagnosis than a classical gene-by-gene approach. Parallel analysis of known genes in a patient (or in multiple patients) enables fast and cost-effective identification of gene mutations. Currently, we are able to perform analysis of 81 genes associated with different inherited skin disorders.
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PS04.27
Molecular genetic testing of FGFR3 gene mutation in the differential diagnosis of achondroplasia and hypochondroplasia in Ukraine

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Introduction: The differential diagnosis of achondroplasia and hypochondroplasia in Ukraine is based on the typical clinical and radiologic features that limits accurate diagnosis and leads to many false-positive diagnoses when checked against a complete mutation search of the FGFR3 gene. Thereby, we outline the necessity of implementation molecular-genetic test of FGFR3 gene mutations. The implementation is necessary to carry out differential and prenatal diagnostic of achondroplasia and hypochondroplasia among Ukrainian population.
Materias and Methods: The study included 61 patients with clinical things of achondroplasia or hypochondroplasia and 94 relatives including sibs and parents. The molecular-genetic analysis was performed by PCR (Polymerase Chain Reaction) and RFLP (Restriction Fragment Length Polymorphism) analysis. We optimized the time and temperature conditions and chose specific primers for revealing the c.1138G>A, c.1138G>C and p.Asn540Lys mutations of FGFR3 gene.
Results: Mutation c.1138G>A was found at 22 (36%) individuals aged from 5 months to 40 years old with obvious phenotypical features of achondroplasia of which 20 cases was sporadic. Additionally the mutation c.1138G>C was detected at 3 (5%) probands. Major p.Asn540Lys mutation of the FGFR3 receptor was identified at 3 (5%) probands which causes hypochondroplasia.
Conclusions: We concluded that 98% of mutations were sporadic because no mutations were found at relatives; meanwhile we found one (1.6%) inheritable c.1138G>A mutation at both mother and son. Due to conducting of molecular-genetic diagnostics of FGFR3 mutations c.1138G>A, c.1138G>C and p.Asn540L diagnosis achondroplasia and hypochondroplasia were confirmed at 26 (43%) observed patients and extensive gene analysis are required for the other patients to search for rare FGFR3 rearrangements.

PM04.28
Inhibition of TGFβ signalling inhibits progression of fibrodysplasia ossificans progressiva in an in vitro model of the disease

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Fibrodysplasia ossificans progressiva (FOP) is a rare congenital disorder characterized by progressive heterotopic ossification. During the disease course FOP patients present soft tissue lumps as a result of inflammation-induced flare-ups preceding the irreversible replacement of skeletal muscle tissue with bone tissue. Classical FOP patients possess a mutation (c.617G>A; R206H) in the activin receptor IA (ACVR1)-encoding gene. Nonetheless, disease progression in FOP patients with this mutation varies which indicates a strong contribution by environmental factors. Our objective was to study the process of osteogenic differentiation in primary dermal fibroblasts from five FOP patients based on a novel method of growth factor-induced osteogenic transdifferentiation. In all patients the classical FOP mutation was confirmed. The osteogenic properties of the cells were evaluated by the mRNA expression of Runt-related transcription factor 2 (Runx2), alkaline phosphatase (Alp), osteocalcin (OC) and the presence of mineralization by alizarin red staining. Given the pro-inflammatory role of TGFβ, we performed pharmacological inhibition of TGFβ signaling by the TGFβ type I receptor inhibitor GW788388. During osteogenic transdifferentiation the expression of Runx2 and Alp over time was higher in FOP cell lines compared to heathy controls (Runx2:p=0.001; Alp:p>0.05). All cell lines exhibited increase in mineralization. Addition of the inhibitor to the osteogenic media resulted in the attenuation of osteogenic differentiation shown by the decrease in expression of osteogenic markers in patients vs untreated cells (Runx2:p=0.045) and mineralisation. We suggest that TGFβ is involved in the molecular pathway of flare-up-induced ossification. Inhibition of this pathway may limit ectopic ossification in FOP.

PS04.29
A rare association of congenital aplasia of the fibula, cleft palate and skeletal abnormalities: a case report

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We report the case of a 35-years old man, referred for genetic counseling because of cleft palate and limb malformations. The family history was unremarkable. He was son of healthy non-consanguineous parents, born at 40 weeks of gestation. Pregnancy was complicated by threatened miscarriage. At birth, clinical evaluation showed cleft palate and presence of multiple malformations of limbs. Hands and foots showed agenesis of some fingers, with syndactyly and complex brachydactyly. Upper limbs were normal. Right femur was hypoplastic with agenesis of the right fibula, brevity and deformation of the proximal epiphysis of the tibia. Other associated anomalies were scoliosis and hypoplasia of the pelvis. Growth and psychomotor development were normal. Complementary investigations including eye fundus, abdominal, renal and cardiac ultrasound examinations and auditory evoked potentials were normal. This syndrome is apparently distinct from other previously described conditions exhibiting fibular agenesis/hypoplasia, because of the association of fibula aplasia/hypoplasia, cleft palate and oligo-brachy-syndactyly of hands and foots is extremely rare. Figuera et al. (1993) reported a girl with fibular agenesis, together with radial shortening and coalescence of the tarsal bones, telecanthus, flat nasal bridge, retrognatia, cleft palate and oligo-polydactyly. This syndrome has been described as "Oral-facial-digital syndrome with fibular aplasia". Some defects are in common with our case, such as the occurrence of cleft palate and fibular aplasia, but in OFD syndrome polydactyly is present, that is absent in our patient. Since this combination has not been described previously, we suggest a distinct new variant of this syndrome.

PM04.30
Genome-wide association identifies a new susceptibility locus at 2q13 associated with clinical vertebral fractures in post-menopausal women

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Clinical vertebral fractures (CVF) are a serious complication of osteoporosis. They are associated with back pain, kyphosis, height loss, cardiorespiratory compromise and increased mortality. Genetic factors play an important role in regulating bone mineral density (BMD) and susceptibility to non-vertebral fractures, but there is little information on the genetic determinants of CVF. Here we report the results of a genome wide association study to identify loci that predispose to CVF, involving 1634 postmenopausal female CVF cases (from 11 centres in Europe and Australia) and 4662 controls, matched for region and gender. Genotyping was performed using the Illumina OmniX array and standard quality control measures were applied. Each cohort was analysed separately and results were combined using inverse-variance meta-analysis. Replication was sought in 634 CVF cases and 2150 controls. We identified seven loci suggestively associated with CVF (p-values=2.02x10-5 - 3.35x10-7) and one locus on chromosome 2q13 significantly associated with CVF (p=2.42x10-8, OR=1.7 [95%CI 1.42-2.09]). The loci are distinct from those previously identified as genetic risk factors for osteoporosis. We went on to study mRNA expression of candidate genes in the 2q13 locus in transiliac bone biopsies from normal and osteoporotic women. Four genes showed reduced levels of expression in osteoporotic patients (ANAPC1, SLC20A1, TMEM87B, ZC3H6) and two showed increased expression (MERTK, ZC3H8). This study has cast new light on the genetic architecture of CVF and identified a novel variant has one of the largest effect sizes ever detected for

fracture risk. Further studies are in progress to identify the causal variants.
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PS04.31

Hemihyperplasia and embryonal tumor- presentation of two cases

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Hemihyperplasia (HH) is an asymmetric body overgrowth due to an unregulated cell proliferation. The frequency of HH might be 1:13500 - 1:86000 newborns. HH may involve one side of the body or single limb or half of the face. Some cases are isolated , inherited dominantly or may be a symptom with variety malformation syndroms- Beckwith-Wiedemann (BWS), Sotos, Silver-Russell (SRS), Proteus. It is well documented that children with HH and/or BWS have an increased risk up to 10% of developing embryonal neoplasms, including nephroblastoma, hepatoblastoma, etc.
Aim. To present two unrelated paediatric patients with typical features of asymmetric body overgrowth, diagnosed HH and embryonal neoplasms.
Case descriptions. The first patient - a girl, body asymmetry was detected at the age of 3 months and as her mother had also HH, autosomal dominant HH was diagnosed and the regular surveillance focusing to embryonal tumors was recommended. Systemic abdominal ultrasound (USD) revealed an adrenal tumor at the age of 22 months, which was successfully operated.
The second patient -a boy, whose HH was diagnosed at the age of 2 months. Abdominal USD was performed at the age of 15 months nephroblastoma was diagnosed and treatment started.
Clinical and pedigree data of the patients are presented, literature data and follow up discussed.
Conclusion. Regular surveillance focusing to embryonal tumors is indicated for children with isolated HH-they have higher risk for embryonal tumor and must be systematically investigated by USD up to six years.

PM04.32

Hereditary Heterotopic Ossification Syndromes: Effect of GNAS inactivation in progressive osseous heteroplasia: A case report

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Bone formation initiates within subcutaneous fat before progressing to deeper tissues in progressive osseous heteroplasia (POH),suggesting that osteogenesis may involve abnormal differentiation of mesenchymal precursors present in adipose tissues. GNAS is a gene encoding two stimulatory G proteins and is a key regulator of fate decisions in adipose-derived mesenchymal progenitor cells that are involved in bone formation. Herein we had detected a patient with GNAS mutation previously not reported.
Case: A 6-year-old boy admitted to our clinic with complaint of left wrist stiffness and limitation in May 2014. His prenatal history revealed oligohydramnious, gestational age as 34 week at birth, delivered with sectio, had 1750 gr birth weight and 45 cm in lenght. At postnatal period he had recurrent infections and apnea . His parents noticed a hard mass on his left wrist when he was 3 months old. The mass progressively became bigger through his first year of life. The biopsy revealed findings as osteoma cutis and could not prove whether it was FOP or POH. In physical examination; he had left wrist and hand deformity, pain with palpation of palmar area. Extension and flexion of left wrist was completely limited, while the range of both supination and pronation were at 30°. He had normal serum PTH, Ca, P, TSH levels. Serial radiographic examinations were reviewed and the progressive HO was noted. The genetic analysis demonstrated GNAS exon 6 mutation as p.Asn167Glnfs*7 (c.498-499insC) heterozygosity and the diagnosis was confirmed as POH certainly. With this case we disscussed the effect of this particular, previously unreported mutation on disease pathogenesis.

PM04.34

EXOME-SEQUENCING APPROACH TO IDENTIFY CANDIDATE GENES FOR INCONTINENTIA PIGMENTI SEVERITY PHENOTYPE

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Incontinentia pigmenti (IP, MIM308300) is a rare X-linked neuroectodermal disorder, caused by mutations in the IKBKG/NEMO gene, regulatory protein of the IKK complex, required for the activation of the NF-kB pathway.
In IP patients the skin defects are hallmarks of the disease, while the extra cutaneous defects (ocular, dental, hair, nail and central nervous system-CNS) are present at variable frequency. In 30% of IP patients CNS defects are observed, and the phenotype severity is variable. Moreover, it does not correlate with the genetic alteration in IP locus. Indeed, even when the common deletion is present (NEMOdel4_10 deletion) a wide range of CNS symptoms can occur, from neonatal seizures to severe mental retardation.
To evaluate the contribution of modifier genes on the variability of the IP intellectual disabilities we investigated the genomic background in three selected IP cases carrying the NEMOdel4_10 deletion and severe mental retardation: one was a familial case and two were sporadic cases. Whole exome-sequencing of trios samples was performed. From sequencing of an exome-enriched library a list of single nucleotide/indels variants was produced. By applying combined filtering method with the information related to the inheritance of the variants, we identified candidate genes by selecting those variants fitting to recessive model of inheritance and damaging by bioinformatics analysis.
Candidate genes were associated to cobalamin and folate pathways suggesting that a masked metabolic defect underlines the IP clinical phenotype. We will present the evaluation of candidate genes acting as modifiers and contributing to the severity of phenotype in IP.

PS04.35

Congenital infiltrating lipomatosis of the face caused by a somatic *PIK3CA* mutation

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Congenital infiltrating lipomatosis of the face is a rare disorder, occuring in infancy or early childhood, in which mature lipocytes invade adjacent tissues of the face. It is characterized by rapid growth, associated osseous hyperplasia and high recurrence rate after surgical intervention. Also premature dental eruption and regional macrodontia have been described. In 2014 Maclellan et al. identified causal missense mutations in *PIK3CA* in affected tissue samples of patients with this disorder.
The phosphatidylinositol-3-kinase (PI3K)/AKT signaling pathway is critical for cellular growth and metabolism and activating mutations in *AKT1, AKT2, or AKT3* have been found in distinct disorders featuring overgrowth. *PIK3CA* encodes the p110α catalytic subunit of PI3K.
Several *PIK3CA* related syndromes with segmental overgrowth have been described before, like MCAP syndrome (Megalencephaly-Capillary Malformations), Hemimegalencephaly, CLOVES syndrome (Congenital Lipomatous asymmetric Overgrowth of the trunk, Vascular malformations, Epidermal nevi, Skeletal and Spinal anomalies) and FH (fibroadipose hyperplasia). Because even more clinical entities were shown to be caused by *PIK3CA* mutations, the name „*PIK3CA*-Related Overgrowth Spectrum (PROS)“ was suggested. Almost all cases are caused by postzygotic (mosaic) mutations in one allele of the *PIK3CA* gene.
We report a patient who presented with asymmetry of the face, with hypertrophy of the right cheek and hemihypertrophy of the tongue. Segmental neurofibromatosis was suspected, but after a biopsy no Schwann cells were found, but only fat cells and fibroblasts, leading to the diagnosis of Congenital infiltrating lipomatosis of the face. DNA analysis of the affected tissue showed a c.1625A>T mutation in the *PIK3CA* gene.

PM04.36

Development of a comprehensive workflow for the analysis of *RYR1* and *CACNA1S* genes associated with malignant hyperthermia; hybridization-based capture and next generation sequencing (NGS) implemented into a routine genetic diagnostics set-up.

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Malignant hyperthermia (MH) is potentially fatal pharmacogenetic disorder in which intracellular calcium homeostasis in the skeletal muscle of susceptible individuals is disrupted upon exposure to halogenated anaesthetics, suxamethonium, or both. MH is linked to the ryanodine receptor (RYR1) on chromosome 19 and the α1S subunit of the voltage dependent L-type calcium channel (CACNA1S) on chromosome 1. Molecular diagnosis of MH is essential for provision of genetic counseling and to establish cascade screening in MH families. We have performed a systematic molecular genetic screening program for MH probands. Many techniques has been implemented and optimized such as DNA/cDNA "*RYR1 hot spot*" sequencing,

melting analysis on Real-Time PCR, MLPA or QF PCR to the routine diagnostics screening algorithm in MH patients. 385 patients from 105 different families underwent molecular genetic testing. In about half of the patients included in our cohort, we identified candidate mutation. There was a need for a more powerful screening tool. A NGS-based workflow was designed using capture library to target the coding and splice site sequences of *RYR1* and *CACNA1S* genes followed by GS Junior pyrosequencing. The developed workflow permitted the identification of MH candidate mutations in 10 of 12 patients examined so far. The workflow meets the sensitivity and specifity requirements for the genetic diagnosis of MH and improves on the cost-effectiveness of current approach. This strategy has been implemented into a routine genetic diagnostics set-up as a first screening approach, potentially before the need of more invasive and specific clinical investigations.

PS04.37

Failure of ossification of the occipital bone : a radiologic handle for diagnosis of mandibuloacral dysplasia type B.

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Mandibuloacral dysplasia type B (MADB) is a rare autosomal recessively inherited disease charectirized by atrophic skin, lipodystrophy and skeletal features. It is caused by mutations in ZMPSTE24 gene encoding a zinc metalloproteinase important in the post-translational modification of lamin. Eleven different mutations in this gene have been identified in 11 patients with MADB from 9 independant families until now.
We report a description of a 12 year-old boy with MADB resulting from a novel missense homozygous mutation in ZMPSTE24 (c.1196A>G ; p.Y399C). The patient had typical skin, sparse hair and skeletal features of MADB, short stature, mild microcephaly, facial dysmorphism and a striking failure of ossification of the interparietal region of the occipital, up to the position where transverse occipital suture can be observed.
Skeletal anomalies such as acrosteolysis, long bone osteolysis, clavicular hypoplasia, spontaneous fractures, delayed closure of cranial sutures, mandibular hypoplasia and skull anomalies are commonly reported in MADB. Wormian bones have described in five cases, but failure of ossification of the occipital bone, reported previously in a single case, appears to be almost pathognomonic for this entity. Occipital bone counts several parts (squama, basio-, exo-, and supraoccipal) and its ossification is multicentric. This observation illustrates that ZMPSTE24 mutations could play a specific role in intramembranouseous ossification of the interparietal part of the squama (Inca bone) but not in the intracartilaginous ossification the supraoccipital. The failure of ossification in the squama appears to be a good handle for radiological diagnosis of mandibuloacral dysplasia type B.

PM04.38

PREDICTIVE PRENATAL TESTING FOR KNOWN FAMILIAL MUTATIONS IN FAMILIES WITH MARFAN SYNDROM

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Objectives:
Marfan syndrome (MFS) is an autosomal dominant connective tissue disorder caused by mutations in the FBN1 gene, resulting in defective glycoprotein fibrillin-1. The major features of MFS involve the cardiovascular, ocular and skeletal systems. Each child of an affected parent has a priori 50% chance of inheriting the mutation. We describe three families with FBN1 mutations which caused classic type of MFS and one family with neonatal form of MFS as a result of maternal germline mosaicism.
Methods:
In the first step a clinically affected future parent was tested. DNA was isolated from peripheral blood and the molecular analysis of FBN1 gene was performed by MLPA and NGS. If the causal mutation was found, fetal samples were taken in the first or second trimester of pregnancy. DNA was isolated from chorionic villus samples or uncultured amniotic fluid. The potential contamination of fetal samples with maternal cells was excluded by characterization of alleles using STR.
Results:
We tested 15 fetal samples whose one parent is a carrier of the causal mutation of the FBN1 gene for classical type of MFS, and 2 fetal samples whose mother is a carrier of the germline mosaicism mutation of the FBN1 gene for MFS neonatal form.
Conclusion:
We have confirmed 9 fetuses with a classic type of MFS and all results were confirmed after their birth. Knowledge of diagnosis is important for their immediate and future health care too. No pregnancy had been terminated following the detection of mutation.

PS04.39

Severe fetal phenotype of a dominant mesomelic dysplasia, associated with a 790 kb microduplication of HOXD gene cluster at 2q31.1

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Objective: The aim of this study was to elucidate the genetic etiology of a very early fetal phenotype of mesomelic dysplasia inherited from the father, and to compare with other phenotypes of mesomelic dysplasia.
Methods: We performed molecular cytogenetic analysis using array CGH and the molecular results were confirmed using QMPSF and FISH.
Results: At 11 weeks of gestation ultrasound showed a cystic hygroma of 4.5 mm, a punctate appearance of the forearm's bones, shortening of the lower limbs, clubfeet, and an unilateral pleural effusion. A TOP was performed at 13 weeks of gestation. Mesomelic dysplasia was confirmed by fetal autopsy, X-rays, and a particular histological appearance(organization of chondrocytes in shortened columns, triangular densification at diaphyseal angulations). The father was followed since childhood for a growth retardation (-2.5 SD) with macrocephaly (+4 SD), ulnar deviation of hands and club feet, a significant shortening of ulna and fibula, bowed forearms and legs, difficulties with flexion / extension of the fingers. Fetal DNA analysis by array-CGH revealed a microduplication of a 790 Kb involving genes *HOXD*13, *HOXD*12, *HOXD*11, *HOXD*10, *HOXD*8, *HOXD*4, *HOXD*3, *MTX*2. This microduplication was also identified in the father.
Conclusions: The different phenotypes of mesomelic dysplasia with early prenatal expression are close, sometimes more severe than the phenotype of an affected parent; research of a genomic microrearrangement is indicated since it can reveal notably a deletion of the *SHOX* region in Xp22.33, or 2q31.1 microduplication encompassing the *HOXD* cluster.

PM04.40

Severe phenotype of metaphyseal dysplasia due to a novel homozygous frameshift mutation in the MMP13 gene

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Introduction: Two sisters of a consanguineous family with Turkish background presenting with short stature and bowlegs were introduced for genetic assessment. The older sister showed more pronounced bowing of the femur and the tibia. Leg pain when walking lead to first surgical correction of both legs at the age of five years. Radiological examination showed genua and coxa vara and metaphyseal and epiphyseal dysplasia of the lower limbs. Additional metaphyseal dysplasia of the radius and the ulna and incomplete closure of the sacral vertebral arch were found concerning the younger sister. Due to suspected recessive inheritance, exploratory genetic testing for shared homozygous alleles was employed.
Material and Methods: Homozygosity mapping was conducted via whole genome SNP analysis using a CytoScan® 750K Array. Subsequent Sanger sequencing of MMP13 gene was performed.
Results: Only one homozygous region (11q22.1-q23.3; HG19:101,897,234-116,462,585) was identified, surprisingly containing the MMP13 gene which is associated with different subtypes of metaphyseal dysplasia. Sequencing of the MMP13 gene revealed a homozygous frameshift mutation (c.381delT) presumably leading to a premature stop codon in the catalytic region of the MMP13 protein.
Conclusion: We describe a novel frameshift mutation in MMP13 gene (c.381delT). Inconsistent with previous reports of inherited metaphyseal dysplasia due to homozygous mutations in MMP13 gene, the associated phenotype of bone dysplasia is severe and requires surgical correction.

PS04.41

Deletion of the miR-17-92 cluster in association with digital anomalies and growth delay

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MicroRNA's (miRNAs) play a key role in the regulation of gene expression. Hemizygous deletions of the MIR17HG gene, encoding the miR-17-92 polycistronic miRNA cluster, are extremely rare. L. de Pontual et al., 2012, reported the first two cases of a germ-line deletion of the miR-17-92 cluster in association with a distinct phenotype consisting of microcephaly, short stature and digital anomalies (Feingold syndrome Type 2, OMIM #614326). We report one further patient with a hemizygous deletion of the miR-17-92 cluster. The patient was referred to our laboratory because of failure to thrive and clinodactyly. Array CGH analysis showed a 648kb deletion of the long arm of chromosome 13 at q31.3 that includes the MIR17HG gene, encoding the miRNA cluster, and one other OMIM gene, GPC5. Real-time quantitative PCR analysis confirmed the result. To date, the mechanism by which the miR-17-92 cluster modulates skeletal development remains uncertain. This case, however, provides further evidence that miR-17-92 plays an important role in normal growth and skeletal development. A full clinical assessment and parental studies will be presented.

PM04.42

Executive function and adaptive behavior in Muenke syndrome

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Objectives: The study investigated executive function and adaptive behavior in persons with Muenke syndrome, the most common craniosynostosis syndrome, using validated instruments with a normative population and unaffected siblings as controls. Additionally, an analysis of modifying factors including absence of craniosynostosis, history of surgery for craniosynostosis, seizures, and hearing loss was done.
Methods: Participants in a cross sectional study included individuals with Muenke syndrome (P250R mutation in FGFR3) and their mutation negative siblings. Participants completed validated assessments of executive functioning (Behavior Rating Inventory of Executive Function; BRIEF) and adaptive behavior skills (Adaptive Behavior Assessment System; ABAS-II).
Results: Forty-four FGFR3 mutation positive individuals, median age 9, range 6 months to 52 years were evaluated with the BRIEF and ABAS-II. Additionally, 10 unaffected siblings were used as controls. For the General Executive Composite scale of the BRIEF, 32.1% of the cohort had scores greater than +1.5 SD, signifying "Potential Clinical Significance." For the General Adaptive Composite of the ABAS-II, 28.2% of affected individuals scored in the "Extremely Low" category" (3rd -8th percentile of normative population) and 53.9% were below the "Average" category (less than the 25th percentile). Multiple regression analysis showed that the presence of craniosynostosis was not a predictor (P = 0.7) of BRIEF and ABAS-II scores.
Conclusion: Individuals with Muenke syndrome are at an increased risk for exhibiting differences in adaptive and executive functioning when compared to a normative population and unaffected siblings. These differences were observed regardless of whether craniosynostosis was present.

PS04.43

Missense substitutions in NF1 and a lack of cutaneous neurofibromas

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Background
Café-au-lait macules (CAL) are the commonest presenting feature of neurofibromatosis type I (NF1), and hence indicators of risk for future NF1-related pathology. Few genotype-phenotype correlations in NF1 are known. Predictors of mild, 'CAL only' phenotypes allow for better prognostication and patient care; p.(992del Met) is the only longstanding example. p.(Arg1809Cys) has recently been reported in association with a similar phenotype (Messiaen, unpublished; Pinna et al, 2014).
Method
We examined genotypes of a cohort of patients with NF1 for evidence of variants predicting a mild phenotype.
Results
Cutaneous neurofibromas (NFs) are less common in patients with missense variants than in those with truncating, frameshift or splice variants ($p<0.05$). p.(Arg1809Cys) is a recurrent substitution (7 individuals from 4 families) in patients without NFs. This mutation has recently been suggested by other groups to be associated with a CAL only phenotype, indicating that this is a robust association. Further substitutions seen in pedigrees without NFs include p.(Arg1276Gln), in 10 individuals from 2 unrelated families, and others clustered around the Ras-binding domain.
Discussion
Missense substitutions in *NF1* predict a lower cutaneous NF burden. It is likely that further specific genotype-phenotype correlations will emerge with testing of more patients. Exclusion of rare complications will require collaborative approaches and long term follow up data.
Conclusion
Certain substitutions in *NF1* segregate with very mild disease. Molecular diagnosis can inform prognosis and management of these patients.
Reference
Pinna et al, Eur J Hum Genet. doi: 10.1038/ejhg.2014.243
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PM04.44

Amplicon-based Next Generation Sequencing: an effective approach to molecular diagnosis of Epidermolysis Bullosa

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Epidermolysis Bullosa (EB) is caused by mutations in genes encoding for proteins of the epidermal–dermal junction assembly. Due to the extreme clinical/genetic heterogeneity of the disease, current methods in EB diagnostics comprise immunohistochemistry on bioptic samples and transmission electron microscopy followed by single candidate gene Sanger Sequencing (SS) that therefore represents the final phase of a labour intensive and expensive clinical pathway.
According to the recently published recommendations for diagnosis and treatment in EB, the assessment of mutational landscape is instead a fundamental step to a comprehensive diagnosis path; Next Generation Sequencing (NGS), throughout parallel ultra-deep sequencing of many genes, would represent a proper method for reducing timing and costs in EB diagnostics.
We developed an EB disease-comprehensive amplicon panel (AmpliSeq panel), to accomplish NGS onto Ion Torrent PGM platform. The panel was dealt on ten patients with known genetic diagnosis, and then employed in eight family trios with unknown molecular footprinting.
The AmpliSeq panel, obtaining a proof of concept of the sensitivity, specificity, and accuracy of this kind of procedure, showed successful in finding the causative mutations in all the ten patients with known mutations, fully confirming SS data. Besides, showing consistent with the clinical diagnosis, it was effective in trios, identifying all the variants, even the ones SS missed or in case of de novo mutations. NGS and AmpliSeq therefore demonstrated to be an effective approach in the diagnosis of EB, resulting in a cost and time-effective 72 hours procedure.
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PS04.45

Targeted NGS for analysis of craniosynostosis identifies a novel mutation in MEGF8

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Craniosynostosis is a frequent craniofacial malformation affecting 1 in 2500 newborns and is defined as the premature fusion of one or more cranial sutures. Premature fusion of the cranial sutures can occur either as isolated malformation in non-syndromic craniosynostoses or as part of a syndrome. So far genetic causes have been identified mainly for syndromic craniosynostoses, i.e. mutations in FGFR2, FGFR3, TWIST1, and EFNB1. However, in more than 50% of cases the underlying genetic cause remains unknown.
We compiled a next generation sequencing (NGS) gene panel comprising 68 genes. In addition to known and candidate craniosynostosis genes of the syndromic and isolated type, the panel includes downstream targets of participating signaling pathways and genes associated with bone development. Target enrichment was performed by the Nextera Rapid Capture Enrichment kit of Illumina and sequencing was done on the Illumina MiSeq

platform. Sequencing data were analysed with the NextGENe software. Performance of the NGS gene panel was validated by sequencing 5 control patients with known mutations. All of these mutations were detected correctly. Subsequently, we sequenced DNA of 13 patients with syndromic as well as isolated craniosynostosis. Two patients are siblings and children of consanguineous parents. Both patients show an a-typical Carpenter phenotype with sagittal craniosynostosis. We identified in both a novel homozygous splice site mutation (c.828G>A) in MEGF8 leading to a predicted loss of the splice donor of exon 5. The mutation was confirmed by Sanger sequencing. Mutations in MEGF8 were shown to be associated with Carpenter syndrome 2 (MIM 614976).

PM04.46

Successful identification of causative single-gene defects using NGS disease-associated genome sequencing approach in a patient affected by perinatal type of hypophosphatasia

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Lethal skeletal dysplasias are highly heterogeneous and phenotypically variable group of genetic condition, often unrecognizable based on the clinical symptoms. Here we report on a foetus affected by severe skeletal dysplasia that resulted in stillbirth at weeks 22 of gestation. The patient presented with generalized limb shortening and intrauterine fractures of the lower leg bones revealed by prenatal ultrasound and autopsy examination. Since no clinical diagnosis could be established, we performed NGS panel encompassing all 2741 genes known to be associated with Mendelian disorders (i.e. disease-associated genome; DAG). With this approach, we were able to demonstrate the diagnosis at a molecular level, which turned out to be perinatal lethal hypophosphatasia (HPP). This severe form of HPP, characterized by an inborn defect of ossification, results either in stillbirth or early postnatal death. NGS panel detected compound heterozygous ALPL missense mutations: c.1283G>C (p.R428P) and c.1363G>A (p.G455S), next confirmed by Sanger sequencing. Mutations demonstrated in our proband, although previously described in other HPP cases, have not been reported to coincide in a single individual. Our study therefore extends the knowledge on HPP and helps in genetic counseling of other patients harboring identical mutations. Importantly, the diagnosis in our index was established by means of NGS-based DAG panel sequencing and would be extremely difficult to reach by any other diagnostic approach. Thus, our report highlights the efficiency and important role of NGS strategies in the diagnosis of prenatally manifesting skeletal dysplasias, especially if the clinical data is insufficient to allow for clinical diagnosis

PS04.47

PRINS, the psoriasis susceptibility related non-coding RNA is involved in stress response of cells, but not in inflammation

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We have previously identified a non-coding RNA, PRINS, as a differentially expressed transcript in psoriatic uninvolved and healthy epidermis. Our in vitro experiment showed that PRINS expression is altered after exposure to various stressors i. e. UVB, translation inhibition and microbial agents. A potential stress signal in psoriatic involved skin may be the extracellular DNA, which activates the AIM2 inflammasome. The activated inflammasome cleaves the precursor proIL-1β form into mature, functioning IL-1β which plays a well-known role in the pathogenesis of psoriasis.

The aim of our study was to investigate whether PRINS affects the expression and activation of IL-1β in normal human epidermal keratinocytes (NHEK) and the elevated expression of PRINS in psoriatic uninvolved epidermis contributes to the inflammatory aspect of the disease.

The highest level of IL-1β secretion could be induced by priming the NHEKs with TNF-α and IFN-γ and a subsequent treatment with the synthetic DNA analogue poly(dA:dT). This treatment resulted in a 2-fold PRINS expression and a significantly elevated (p=0.02) IL-1β secretion. Next we silenced and overexpressed PRINS in NHEKs and performed the above IL-1β inducing treatments. Neither the silencing nor the overexpression of PRINS had any effect on the IL-1β secretion of NHEKs suggesting that this non-coding RNA does not have any regulatory role in the AIM2 inflammasome signaling.

PM04.48

Olmsted syndrome: a novel homozygous TRPV3 mutation with severe phenotype

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Olmsted syndrome (OLMS) (MIM#614594) is a rare, severe keratinization disorder characterized by bilateral mutilating palmoplantar keratoderma and periorificial keratotic plaques. Other associated features include corneal lesions, diffuse alopecia or sparse hair, digital constriction, nail dystrophy, autoamputation of fingers and/or toes. Infections and squamous cell carcinomas can arise on the keratotic areas. OLMS should be differentiated from several genodermatoses, such as Vohwinkel syndrome and Acrodermatitis enteropathica. Although most cases are sporadic, both autosomal dominant and X-linked inheritance have also been reported. The heterozygous mutations in TRPV3 were identified as a cause of autosomal dominant type, while the X-linked recessive inheritance was associated with the mutations in MBTPS2. Recently, the homozygous TRPV3 mutations have also been reported in a few recessive cases.

We report a 16-year-old male patient who has referred to our clinic with severe palmoplantar keratoderma, autoamputation of fifth toes, mild periorificial, perianal and genital keratotic plaques, and mild intellectual disability with prediagnosis of OLMS. His parents had consanguinity and his brother, who has died at the age of 8 due to complication of infections, had similar phenotype. We performed whole exome sequencing (WES) for the index case and a novel homozygous TRPV3 mutation (NM_145068;c.1247G>T;p.Arg416Leu) was identified in our patient. Additionally, we confirmed this mutation by using Sanger sequencing and the parents were found to be heterozygous carriers for this mutation. To our knowledge, this is the third reported autosomal recessive OLMS case in the literature. We conclude that the homozygous TRPV3 mutations result in a more severe OLMS phenotype.

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PS04.49

Nonsyndromic cleft lip with or without cleft palate: Genome-wide imputation identifies four novel risk loci and an enrichment of association signals in enhancer datasets relevant to craniofacial development

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Nonsyndromic cleft lip with or without cleft palate (nsCL/P) is a common congenital malformation and has a multifactorial etiology. Currently, 15 nsCL/P susceptibility loci have been established. Some of the yet unidentified genetic risk factors might be detected by increasing the marker density in genome-wide datasets. We used data from a genome-wide meta-analysis of European individuals (Ludwig et al. 2012, Nature Genetics). Based on ~500.000 genotyped markers, imputation was performed using 2184 alleles (1kGP data). After quality control, a high-density dataset of ~8.26 million variants was statistically analyzed.

One previously unreported SNP was identified with genome-wide significance (rs6740960 at 2p21, P=1.6x10-08). Interestingly, this variant maps 5bp adjacent to a predicted Myc binding site upstream of the PKDCC gene. Pkdcc is downregulated in mice carrying a deletion of the region homologous to the 8q24 nsCL/P major risk locus (Uslu et al. 2014). Additionally,

SNPs at 64 loci showed suggestive evidence of association. Replication at 29 loci in an independent nsCL/P sample (609 cases, 1.745 controls) was performed. After combined analysis, three additional loci reached genome-wide significance: 2p24.2, 14q22.1 and 15q13.3. At genome-wide level, we observed a significant overrepresentation of association signals in functional datasets relevant to craniofacial development such as enhancer datasets from human neural crest cells (Rada-Iglesias et al. 2012) and murine embryonic craniofacial tissue (Attanasio et al. 2013).
Our study identified four novel risk loci for nsCL/P and revealed an over-representation of association signals in functionally relevant datasets. Ongoing analyses include assessment of heritability estimates, and ingenuity pathway analyses.

PM04.50
Antenatal detection of intracranial calcification in a baby with osteocraniostenosis

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Osteocraniostenosis (OCS) is a rare, perinatally lethal skeletal dysplasia characterised by gracile bones, premature closure of basal cranial sutures and microphthalmia. Recently, heterozygous mutations in the gene *FAM111A* have been shown to cause OCS.
OCS is allelic to Kenny-Caffey syndrome (KCS), which has a less severe phenotype of small, dense bones, short stature and primary hypoparathyroidism with hypocalcaemia. *FAM111A* mutations have been identified in both disorders. Recent publications suggest that *FAM111A* plays an important role in a pathway controlling calcium homeostasis.
We present a case of an infant who was found antenatally to have a cloverleaf skull, reduced bone density and intracranial calcification (presenting as intracranial echogenicity on ultrasound scanning). The presence of intracranial calcification led to a relatively long list of differential diagnoses. The baby was born at term but died within a few hours of birth. On post mortem examination features of OCS were identified and there was confirmation of foci of calcification in the basal ganglia. A *de novo* heterozygous mutation in *FAM111A* was subsequently detected, confirming the diagnosis.
Intracranial calcification is a recognised feature of OCS, but only on postnatal imaging. Antenatal detection of intracranial abnormalities has not previously been reported. We report the clinical and radiological findings of this rare skeletal dysplasia, review the literature and suggest that OCS should be in the differential diagnosis of antenatal intracranial calcification.

PS04.51
Novel form of recessive osteogenesis imperfecta caused by missense mutations that alter a collagen type I-interacting protein

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Osteogenesis imperfecta (OI) is a heritable bone fragility disorder that is most often caused by mutations in *COL1A1* (MIM 120150) or *COL1A2* (MIM 120160). Defects in sixteen other genes have been identified in association with dominantly and recessively inherited forms of OI. These genes play a role in the processing of collagen type I or in the control of osteoblast differentiation or function. Despite these advances, a small proportion of individuals that present with a phenotype of severe OI do not have mutations in any of the genes that are known to be associated with OI. We performed whole-exome sequencing to identify the molecular defect in two unrelated girls with severe bone fragility and a clinical diagnosis of osteogenesis imperfecta type IV, and identified two homozygous variants that affect very conserved and interacting residues in a novel protein that interacts with collagen type I and other matrix proteins. The residues substituted by these mutations are essential for the binding of this protein to collagen type I. Skin fibroblasts from our patients express the protein at apparently normal levels but secretion of collagen type I was delayed. Binding of the mutated protein to collagen type I was decreased. Analysis of an iliac bone sample from one affected individual showed that the trabecular bone was hypermineralized on the material level. Herein we present a detailed clinical, radiological and molecular description of this novel form of autosomal recessive OI.

PM04.52
Osteoblasts from Type V OI patients demonstrate gain-of-function for mineralization despite decreased COL1A1 expression

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Osteogenesis imperfecta (OI) is a genetically heterogeneous disorder characterized by bone fragility. OI is now understood to be a collagen-related disorder, with mutations in type I collagen structure causing dominant OI, while deficiency of collagen-related proteins cause rare recessive OI forms. Type V OI is the only rare OI type to also have dominant inheritance. It is caused by a heterozygous mutation (c.-14C>T) in IFITM5, which encodes BRIL, a transmembrane protein expressed in osteoblasts. The mutation generates a start codon, adding five residues to the BRIL N-terminus. However, the mechanism of type V OI and its relationship with type I collagen is unknown. We identified 8 patients with type V OI. Using cultured patient osteoblasts, we verified normal expression and stability of mutant IFITM5 transcripts and BRIL protein level. Both early (ALPL and IBSP) and late (osteopontin and osteocalcin) markers of osteoblast differentiation are increased in type V OI osteoblasts. Mineralization, assayed by alizarin red staining, was increased in type V OI osteoblasts vs control. In contrast, type V OI osteoblasts have one-third the level of COL1A1 transcripts in control in mid to late differentiation, with concomitantly decreased collagen protein secretion, crosslinked collagen in matrix, and altered appearance of fibrils deposited in culture. The increased mineralization and advanced differentiation of type V OI osteoblasts demonstrate a gain-of-function mutation leading to the overactive tissue calcification and hypertrophic callus formation seen in type V patients. Decreased type I collagen expression, secretion and matrix incorporation establish type V OI as a collagen-related defect.
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PS04.53
Mutational analysis in Osteogenesis Imperfecta patients

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Osteogenesis imperfecta (OI) is a clinically and genetically heterogeneous brittle bone disorder. The aim of our study was to identify mutations in COL1A1, COL1A2, CRTAP, LEPRE1, PPIB and SERPINF1 genes in Russian OI patients.
We examined 78 patients with OI and 100 healthy controls corresponding by age, gender, ethnicity and place of residence. We sequenced the coding and exon-flanking regions of COL1A1, COL1A2, CRTAP, LEPRE1, PPIB and SERPINF1 genes.
We identified 7 distinct mutations, undescribed before.
For the first time previously unreported nonsense mutation c.967G>T (p.Gly323X), 2 novel frameshift mutations c.3541insC (p.Gly1181ArgfsX38) and c.1098_1099insA (p.Gln367ThrfsX5) and one splicing mutation c.3208+1G>C in COL1A1 gene was observed in 7 patients,
The splicing mutation c.1724+4G>A in LEPRE1 gene was identified in two patients. And novel compound heterozygous mutations (c.913C>G (p.Leu305Val) of SERPINF1 gene and c.641T>C (p.Val214Ala) of CRTAP gene) was observed in three patients.
We also detected three previously described nonsense mutations in seven Russian patients: c.1081C>T (p.Arg361X), c.1243C>T (p.Arg415X) and c.2869C>T (p.Gln957X), two frameshift mutations in two patients: c.579delT (p.Gly194ValfsX71) and c.2444delG (p.Gly815AlafsX293) and two splicing mutations in three patients: c.4005+1G>T and c.697-2A>G.
In conclusion, the present study revealed 11 mutations in COL1A1 gene, 1 mutation in SERPINF1 gene, 1 mutation in LEPRE1, 1 mutation in CRTAP gene, 7 of them was not observed before and no mutations in COL1A2 and PPIB genes in Russian patients with OI. Future research will focus on other genes responsible for OI development in Russian patients

PM04.54

Targeted sequencing of the Paget's disease associated 14q32 locus identifies several missense coding variants in RIN3 that predispose to Paget's disease of bone

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Background: Paget's disease of Bone (PDB) is a common skeletal disorder with a strong genetic component. We identified a susceptibility locus for PDB on chromosome 14q32 by GWAS, tagged by rs1049863 within the RIN3 gene. Here we investigate the candidacy of RIN3 as a predisposing gene for PDB.
Methods: We conducted re-sequencing of the 14q32 locus in PDB cases and controls and studied expression of RIN3 in bone tissue and bone cells by quantitative PCR, western blotting and immunohistochemistry.
Results: We detected 16 missense variants in RIN3, including 12 rare potentially damaging variants, 7 of which were detected only in PDB. A common coding variant (p.R279C) in LD with the GWAS hit (r2=0.96) was strongly associated with PDB (OR=0.64, P=1.4x10-9). The rare variants were also strongly associated when combined (OR=3.72; P=8.9x10-10). mRNA for RIN3 was strongly expressed in lung, bone and liver with increased expression in osteoclasts as compared with bone marrow (p=0.02). Expression of RIN3 increased at the mRNA and protein level during osteoclast differentiation in vitro.
Conclusions: These findings indicate that RIN3 is the causal gene for PDB on 14q32 and suggest that its susceptibility is mediated by a combination of common and rare coding variants. While the function of RIN3 in bone biology is incompletely understood the data are consistent with a model whereby RIN3 acts to inhibit osteoclast formation and that the predisposing variants damage its ability to do so, resulting in the osteoclast activation characteristic of PDB.
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PS04.55

DESCRIPTION OF A NEW POLYMORPHISM IN SQSTM1 GENE IN PATIENTS WITH PAGET´S DISEASE OF BONE

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Paget's disease of bone (PDB) is a focal disorder of bone that affects segmentally the skeleton, presenting an increase in osteoclast number, size and activity that results in a variegated and anarchic bone structure that alters the mechanical properties.
The existence of genetic factors is one of the etiopathogenic hypothesis that attempts to explain the origin of PDB. The most plausible candidate gene is sequestosoma1 gene (SQSTM1), encoding the p62 protein, which plays an important role in cellular signal crossroads related with osteoclastogenesis
Only 3% of patients in a historical cohort of 288 patients were carriers of a mutation in SQSTM1 gene. Five patients were carriers of the c.1000G>C (p.E273D) mutation, not described to date in the literature.
The aim of our study was to try to evaluate the putative role c.1000G>C (p.E273D) mutation in the development of PDB.
"In silico" study categorizes c.1000G>C mutation as pathogenic. A population study in 100 healthy individuals alleles by dHPLC failed to detect mutation.
Then, the cDNA of SQSTM1 gene was cloned in the expression vector pCEFL-FLAG. After site directed mutagenesis the c.1000G>C mutation was incorporated into the construction. Finally COS1 cells were transfected with our construct to perform western blotting and immunofluorescence assays.
By immunofluorescence and western blot assays we did not find differences between the transfected cells with the wt construct and transfected cells with the c.1000G>C construct (Fig1-2).
In conclusion, our results suggest that c.1000G>C (p. E273D) mutation should be considered as a neutral mutation, a polymorphism that increases risk to suffer PDB.
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PM04.56

Molecular basis of aggressive periodontitis

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Chronic periodontitis is an inflammation disorder affecting 30-50% of the adult population, leading to teeth attachment loss and degradation of the alveolar bone. Aggressive periodontitis (AP) is a rare form of the disease with a prevalence of approximately 0,1%. It is characterized by an early age of onset, rapid rate of progression and high inflammation activity leading to loss of teeth before the age of 35, if untreated.
Here we report on a Tyrolean four-generation family indicating an autosomal dominant inheritance pattern for AP. The result of our genome-wide linkage analysis revealed one large co-segregating chromosomal region in affected individuals. The interval is 12 Mb in length and contains approximately 300 genes. In addition, we performed whole exome sequencing in four affected family members to identify possible common variants in the linkage region. Assuming a monogenic cause for the disease we have been looking for an unknown, heterozygous mutation in the coding sequence and exon/intron boundaries. Identified variants were filtered with common parameters including alignment against various mutation databases and prioritized based on their translational and predicted functional effects.
In our functional studies we have already shown a significant reduction of chemotactic and phagocytic activity of neutrophils in affected individuals. In regard to
these preliminary results we propose a mutation contributing to an immunological dysfunction. Signs of Ehlers-Danlos-syndrome (EDS) present in some family members may also suggest a mutation resulting in disturbed connective tissue deposition.

PS04.57

A zebrafish model for Bruck Syndrome caused by PLOD2 mutations

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Bruck syndrome, a disorder caused by recessive mutations in either *PLOD2* or *FKBP10*, is characterized by contractures and bone fractures and shows strong clinical overlap with Osteogenesis Imperfecta (OI). Animal models for OI are indispensable for unraveling molecular mechanisms in OI pathogenesis. The zebrafish was recently shown to be a useful vertebrate organism to model OI both at the phenotypic and molecular level. Zebrafish mutants that model OI display generalized reduced bone density and misshapen bones with evidence of fractures. Although the molecular role of *PLOD2* has been documented, no animal models for Bruck syndrome, caused by *PLOD2* mutations, have been reported. To elucidate the function of *PLOD2* in vertebrate skeletal development, zebrafish harbouring a homozygous *plod2* nonsense mutation were phenotyped using µCT scanning, alizarin red staining for bone, toluidine blue staining and ultra-thin sectioning. Mutants presented with a shortened body axis and malformed craniofacial structures. The skeleton was severely affected with evidence of bone fragility and fractures, bowing and kinking of the ribs and fin bones. The vertebral column was scoliotic with compressed vertebrae and excessive periosteal bone formation at the vertebral end plates. The observed phenotype is concordant with the clinical findings detected in Bruck Syndrome patients. Therefore, the *plod2* zebrafish mutant is a promising model for elucidation of the underlying pathogenetic mechanisms leading to Bruck Syndrome.
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PM04.58

Whole Exome Sequencing as a novel tool for the detection of modifier genes in Pseudoxanthoma elasticum

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Pseudoxanthoma elasticum (PXE), an autosomal recessive ectopic mineralization disorder caused by ABCC6 and ENPP1 mutations, is characterized by skin, ocular and cardiovascular (CV) symptoms. PXE shows distinct clinical variability, with growing importance of modifier genes. Modifiers have previously been studied using a candidate gene approach, which only yielded VEGFA as a modifier of the PXE retinopathy.
We introduced Whole Exome Sequencing (WES), capable of analyzing multiple genes in a single reaction, to identify potential modifiers by comparing patients with extreme phenotypes. WES was performed in 13 molecularly and histologically confirmed PXE patients with an extreme (absent or severe) CV phenotype (based on clinical presentation and vascular calcium scoring).
Comprehensive functional analysis of variants unique to the severe cohort led to 9 modifier candidates, which were subsequently validated using Sanger sequencing and screened in an independent cohort of 50 PXE patients. Genotype/allele frequency analysis and multiple logistic regression confirmed significant association of 3 SNPs with severe CV disease: rs2228570 (VDR gene), rs13006529 (CASP10 gene) and rs1042714 (ADRB2 gene). All were previously linked to CV disease and functional data mining yielded links to the PXE pathophysiology. Particularly for rs2228570, reported to increase VDR transcriptional activity, upregulation of several VDR transcriptional targets was demonstrated in PXE.
In conclusion, WES enables to characterize candidate modifier genes through a targeted analysis approach in patients with extreme phenotypes. Our results suggest a role for VDR, CASP10 and ADRB2 in determining the severity of CV complications in PXE, providing valuable assets for management of PXE families. FWO14/ASP/084

PS04.59
Co-inherited ENPP1 mutations can intensify the cardiovascular phenotype of pseudoxanthoma elasticum

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Pseudoxanthoma elasticum (PXE) is characterized by aberrant mineralization and fragmentation of elastic fibers, leading to skin, ocular and cardiovascular symptoms with a significant variability in severity. The molecular etiology of PXE has become increasingly challenging as mutations in both ABCC6 and ENPP1 were shown to cause overlapping phenotypes and modifier genes are beginning to be identified. We evaluated the causal role of ENPP1 mutations in PXE patients with an incomplete ABCC6 genotype in both an autosomal recessive or digenic inheritance model. Further, we assessed whether ENPP1 variants, which also cause a severe cardiovascular disease called Generalized Arterial Calcification of Infancy (GACI), could have a disease modifying effect in PXE patients with an exceptionally severe and rapidly progressive (cardio)vascular phenotype. ABCC6 and ENPP1 genotyping of 40 clinically and histologically confirmed PXE patients was performed by Sanger sequencing and MLPA. A vascular calcium score was obtained through whole body CT scanning. In a cohort of 40 PXE patients (13 with an unidentified and 17 with an incomplete ABCC6 genotype; 10 with severe cardiovascular disease), no additional ENPP1 mutations could be found. In one patient of the cardiovascular cohort, an additional ENPP1 mutation co-inherited with biallelic ABCC6 mutations was identified. The vascular calcification score of this patient was exceptionally high (Agatston score of 3600 compared to an average of 422 in PXE patients), resembling the findings in GACI patients. We demonstrate that the co-inheritance of ENPP1 mutations together with ABCC6 mutations has an intensifying effect on the cardiovascular phenotype of PXE.

PM04.60
Development of a Genetically-Modified Human Dermal Fibroblast for the Treatment of Recessive Dystrophic Epidermolysis Bullosa (RDEB)

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Recessive dystrophic epidermolysis bullosa (RDEB) is an autosomal recessive, inherited skin disease caused by null mutations within the type VII collagen gene (*COL7A1*). The mutations cause an absence or reduction of functional collagen VII, which make up anchoring fibrils that maintain binding of the epidermis to the dermis. The disease is characterized by a mechanical fragility and repeated blister formation in the sub-lamina densa, at the level of the structurally defective anchoring fibrils. Currently, there is no effective therapy for this disease, and death is usually the result of aggressive squamous cell carcinoma, sepsis, or malnutrition.
We are developing an autologous, genetically-modified fibroblast cell therapy that is anticipated to improve skin function in RDEB patients through restoration of collagen levels. A patient's fibroblasts will be harvested, genetically modified *ex-vivo* with a functional *COL7A1* gene, and expanded in culture (GM-HDF-COL7). *Ex vivo* transduction will occur through the use of a replication-defective, self-inactivating (SIN) lentiviral vector. After expansion, the fibroblasts are administered back to the patient as a local intradermal injection into target wound margins. The resulting increase in anchoring fibrils is anticipated to stabilize the connection between skin layers and reduce blistering tendency.
In vitro product development data indicates that cGMP scale GM-HDF-COL7 cells express full-length type VII collagen exhibiting the proper trimeric structure, size, and binding functionality. A hybrid *in vitro/in vivo* pharmacology/toxicology study using an organ culture/SCID mouse model is underway at Stanford University to confirm type VII collagen persistence, distribution, localization and toxicology.

PS04.61
Patients with isolated oligo/hypodontia caused by *RUNX2* duplication

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Loss-of-function mutations of *RUNX2* are responsible for cleidocranial dysplasia, an autosomal dominant disorder characterized by delayed closure of cranial sutures, aplastic or hypoplastic clavicles, moderate short stature and supernumerary teeth. In contrast, an increased gene dosage is expected for duplication of the entire sequence of *RUNX2* and, thus, a different phenotype as cleidocranial dysplasia. To date, two cousins with a duplication including the entire sequence of *RUNX2* in addition to *MIR586*, *CLIC5* and the 5' half of the *SUPT3H* gene have been reported. The patients presented with the association of metopic craniosynostosis and hypodontia.

We report here a family with four patients carrying a 285 kb duplication including the entire sequence of *RUNX2* and the 5' half of the *SUPT3H* gene. Two patients presented with the association of metopic craniosynostosis and oligo/hypodontia previously described, confirming the phenotype caused by a duplication of the entire sequence of *RUNX2*. Interestingly, the two other patients had isolated hypodontia without any craniosynostosis, enlarging the phenotype observed in patients with such duplications.

PM04.62
Childhood osteoporosis and enamel dysplasia: expanding the spectrum of duplication in RUNX2

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RUNX2 gene encodes CBFA1, a master transcription factor, regulating both osteoblasts and terminal chondrocytes differentiation, is essential for bone development and mineralization.
A 105-kb duplication containing exons 3-5 of the RUNX2 gene was recently found in affected members from a family with Metaphyseal Dysplasia and Maxillary Hypoplasia with or without Brachydactyly (MDMHB, OMIM 156510). Transfection studies with murine dup3-5 Runx2 cDNA showed a higher transactivation activity suggesting of gain of function of the gene in patients with MDMHB, contrary to the RUNX2 haploinsufficiency, which is associated with Cleidocranial Dysplasia (CCD, OMIM 119600), defined by persistent open skull sutures, clavicles hypoplasia and dental anomalies.
MDMHB is described in only 3 families (2 Canadian and 1 Finnish), and is characterized by metaphyseal flaring, wormian bones, mild osteoporosis, enlargement of the proximal portion of the clavicles, and yellowish teeth. We have identified 3 children from 2 unrelated families (Cambodia and Algeria), presenting with clinical features resembling to MDMHB. They were all referred for Osteogenesis Imperfecta. CGH array and Q-PCR detected a RUNX2 duplication spanning exon 2 to exon 6, resulting, as in the MDMHB Canadian family, to the duplication of the functional CBFA1 QA and RUN domain. In both families, RT-PCR detected interestingly that the fathers were mosaic for this RUNX2 duplication. We highlight the main features of this recognizable phenotype of childhood osteoporosis with abnormal teeth. Finally, we discuss the RUNX2 threshold for consequences on the formati-

on of endochondral and intramembranous bone tissue and odontogenesis process.

PS04.63

Variants of the matrix metalloproteinase-3 gene are not associated with anterior cruciate ligament ruptures

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Introduction: DNA sequence variants have been associated with the risk of musculoskeletal soft-tissue injuries, including several mapping to the matrix metalloproteinase-3 gene (*MMP-3*). This gene encodes an endopeptidase, which by function, degrades extracellular matrix components. Three *MMP-3* variants namely; rs679620, rs591058 and rs650108 have previously been associated with Achilles tendinopathy. Moreover, variants; rs679620 and rs3025058, have been studied for their association with anterior cruciate ligament ruptures (ACL) in two studies. Therefore, the aim of this research was to explore all four variants within *MMP-3* with the risk of ACL injuries.
Material and Methods: A case-control genetic-association study was employed. In total, 232 control participants (CON) and 234 participants with ACL ruptures (ACL) were genotyped for all four *MMP-3* variants. Within the ACL group, 120 participants reported a non-contact mechanism of injury (NON subgroup). Investigated polymorphisms included; rs3025058 (5A/6A), rs679620 (G/A), rs591058 (C/T) and rs650108 (G/A) respectively. Statistical analysis involved investigating the allele and genotype frequency distributions in addition to conducting inferred haplotype analysis between the cases and control participants. Significance was accepted when $P<0.05$.
Results: After adjusting for age, sex and weight, no independent associations were noted for all genotype frequencies ($>P=0.350$). Furthermore, no differences in the inferred haplotypes amongst the cases and controls were observed. All variants were in Hardy-Weinberg Equilibrium ($P>0.05$).
Conclusion: These preliminary observations are in contrast to the previous findings and therefore this genomic region requires further interrogation.
Grants: Research supported by NRF and UCT. Andrea Gibbon funded by NRF and UCT. Michael Posthumus funded by the Thembakazi Trust.

PM04.64

Analyses of the molecular pathways involved in the sarcopenic process in functional and non-functional elderly population.

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Abstract
Sarcopenia is one of the most deleterious effects of aging. The involuntary loss of muscle mass, strength, and muscular function have a major impact on quality of life in the elderly population. The etiology of the sarcopenia is not clearly established, although, a multifactorial process that develops from the fourth decade of life is proposed. In a previous clinical report we found that evaluation of the muscular contractility is a *per se* and independent factor in the sarcopenia development not related with the muscle volume. In order to understand this phenomenon, we analyzed the molecular pathways involved in the muscle performance and the sarcopenic process. Genetic expression patterns in functional and non-functional elderly groups were studied in 15 muscle biopsies from both groups, using a QRTPCR array (RT2Profiler, Qiagen). We studied critical signaling pathways involved in the sarcopenic process (apoptosis, autophagy, WNT, TGF-Beta, TNF-Alpha and interleukins). Immunohistochemestry and confocal analysis were also performed in order to explore differences in the distribution of the muscular proteins involved in the contractile process (actin, myosin, myopalladin and SERCA). Preliminary results showed an overexpression of genes involved in apoptosis, autophagy and interleukins in the non functional group. Confocal analysis showed a different protein distribution in a qualitative a quantitative fashion in both groups. Our data suggests that inflammatory and apoptotic process in the muscular fiber impacts the proteins involved in contractility and muscular functionality, observed in the clinic as less contractility, strength and muscular performance in the sarcopenic and non functional population.
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PM04.66

JmjC demethylase mutation and Split Hand/Foot Malformation with Long Bone Deficiency.

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We studied an Italian nuclear family with three individuals (mother and two of the siblings) presenting Split Hand/Foot Malformation with Long Bone Deficiency (SHFLD). The duplication at 17p13.3, encompassing bHLHA9 gene and commonly associated with this disease, segregated within the family. The duplication was detected in a set of healthy subjects originating from Southern Italy, suggesting that it could be merely a susceptibility factor. Consistent with this hypothesis, the bHLHA9 duplication was found associated with a range of other limb malformations.
To possibly identify the causative genetic lesion we exome sequenced all members of the family. We filtered, consistently with the expected autosomal dominant inheritance, a heterozygous nonsense mutation (c.4000C>T/p.R1334X) in the KDM5B gene that co-segregates with SHFLD and targets transcripts to nonsense-mediated mRNA-decay, contributing to protein haploinsufficiency. KDM5B encodes a JmjC demethylase, which regulates genes with a role in bone metabolism. The expression of two of its targets, SMAD7 and TCF3, is modified in KDM5B haploinsufficient cells. We similarly showed through luciferase assays that wild-type KDM5B, conversely to the truncated form, controls the promoter of RUNX2, a key transcription factor involved in osteoblastic differentiation. Moreover the expression levels of RUNX2 and OPG are altered in a model of osteoblast-like cells, established from patient's skin fibroblasts. Overall these results suggest that haploinsufficiency of KDM5B could cause transcriptional alteration of genes linked to bone development.
Therefore, considering the variable expressivity and reduced penetrance of SHFLD, we propose KDM5B as a likely second genetic event participating in the index family.

PS04.67

Silver-Russell syndrome phenotype in a patient with a methylation abnormality associated with Beckwith-Wiedemann syndrome

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Introduction: Beckwith-Wiedemann syndrome (BWS), an overgrowth disorder, and Silver-Russell syndrome (SRS), a growth retardation disorder, are imprinting disorders associated with abnormalities of the 11p15 region. The majority of BWS cases result from loss of methylation at the KvDMR imprinting control centre whilst ~40% of SRS cases arise from loss of methylation at the H19 DMR imprinting control centre.
Materials and methods: 11p15 MS-MLPA was performed to detect dosage and methylation abnormalities of the 11p15 region in a patient with a suspected diagnosis of SRS.
Results: The patient was found to have loss of methylation at KvDMR at all probes tested and no 11p15 dosage abnormalities. Loss of methylation at KvDMR is a known cause of BWS but not SRS.
Conclusion: This is the first report, to our knowledge, of a patient with loss of methylation at KvDMR only presenting with a SRS phenotype. There have been previous reports of rare patients with loss of methylation at both KvDMR and H19 DMR that present with SRS. It has been hypothesised that the region with greater demethylation gives the dominant phenotype and that, in cases where multiple regions have a similar degree of methylation, one region might have a dominant effect. Also ~45% of SRS cases have an unknown etiology. Therefore, a possible reason that this patient is presenting with a SRS phenotype despite having loss of methylation at KvDMR only, could be that another, as yet unknown, locus with a dominant effect is also affected.

PM04.68

High success of a next generation sequencing panel in the genetic analysis of rare skeletal dysplasias

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In 2010, 456 skeletal dysplasias (SD) were classified by molecular, biochemical and/or radiological criteria, into 40 groups. The precise, final diagnosis is often difficult due to the high phenotypic and genotypic variability. Aim: To improve SD molecular diagnosis using a custom-designed Next-generation sequencing (NGS) panel, including a total of 315 genes or regulatory regions (967Mb).
A total of 60 SD probands without known mutations were clinically and radiologically evaluated. All probands and in some cases family members, were analyzed using the SKELETALSEQ.V3 panel and sequenced (16 patients/run) on a MiSeq (mean coverage 100X). All variants were confirmed by Sanger sequencing or aCGH.
The molecular defect was identified in 24 probands (40%): 23 mutations (missense, stop or small deletions) and one GLI3 deletion. Interesting cases include the identification of the first FGF9 mutation in a family with craniosynostosis, a second case of spondylometaphyseal dysplasia due to POP1 mutations and the detection of POCA1 mutations in two families with primordial dwarfism/SOFT syndrome.
In conclusion: 1) The SD-NGS panel has permitted a high mutation detection rate, 40%, higher than in other pathologies (average 26%). 2) We have demonstrated that FGF9 mutations can cause not only multiple synostosis but also sagital/coronal craniosynostosis in humans, as observed in the spontaneous mouse model. 3) The advancement in genetic diagnosis of these disorders will improve the management, monitoring and treatment of the patients and allow prenatal and preimplantational studies. 4) Exome sequencing may be helpful in those cases in which no mutation was identified.

PS04.69

An interstitial deletion of chromosome 7q35q36.1 in a 3-year-old boy with clinical features of Weaver syndrome

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The phenotype of EZH2-related overgrowth known as Weaver syndrome usually incorporates tall stature, variable intelligence, advanced bone age (100%), poor coordination, soft doughy skin, camptodactyly, umbilical hernia, a hoarse low cry in infancy. Facial characteristics may contain macrocephaly, broad forehead, round face, large, fleshy ears, hypertelorism, almond-shaped eyes and retrognathia. Most cases of Weaver syndrome are sporadic and caused by a heterozygous mutation in the EZH2 gene on chromosome 7q36.
The aim is to present a case report of a 3-year-old boy first investigated because of speech delay. His birth weight was 5080 g (> + 2 SD), length 53 cm (0 SD) and head circumference 39 cm (> + 2 SD). In early neonatal period he had an episode of hypoglycemia. At the age of 3 years his head circumference was rather large (+2 SD) but his height was normal (102 cm, +0.5 SD). He had mild dysmorphic features - round face, large, fleshy ears with speech delay, which could be the statement of intellectual disability. Chromosomal microarray analysis (Human CytoSNP-12 BeadChip, Illumina Inc.) revealed a 3Mb de novo interstitial deletion on paternal chromosome 7 in bands 7q35q36.1. The region contained 2 disease-related genes: CNTNAP2 and EZH2.
Conclusion: In this case we present the patient who has some clinical features of Weaver syndrome caused by 7q35q36.1 microdeletion. This is a very rare microdeletion and at the moment its prevalence is unknown.

PM04.70

Prenatal detection of likely causal 4q28.3q31.21 microdeletion in a foetus with skeletal dysplasia.

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We report a newborn boy (37th WG, Caesarean section) prenatally monitored for multiple ultrasound anomalies. 37 year old woman underwent first prenatal ultrasound examination at her first pregnancy at 13th WG. Considerable shortening of the lower limbs and spine kyphosis were found and chorionic villi sampling with a subsequent SNP array (300K, Illumina) and FGFR (OMIM*146000) gene mutation screening (negative) were done.
SNP array revealed 4.8 Mb long heterozygous microdeletion at 4q28.3q31.21 that spanned 14 OMIM genes. SNP array examination of the parents showed 0, 87 Mb long microdeletion at 4q28.3 in the phenotypically normal father. Father's microdeletion was shorter and compared to the foetus microdeletion did not span 13 OMIM genes. One of the genes deleted only in the foetus was the RAB33B gene (OMIM*605950), in which the recessive mutations were recently identified in individuals with skeletal Smith-McCort Dysplasia 2 (SMC2, OMIM#615222). Although the phenotypic signs in the foetus were not typical for SMC2, microdeletion was considered to be likely pathogenic for the foetus. Results were discussed with the couple and the parents decided to continue pregnancy. Repeated ultrasound examination at 16th WG confirmed significant femurs shortening, thoracic kyphosis and mild shortening of the upper limbs. Ultrasound at 27th WG showed apart from the skeletal dysplasia ventricular septal defect, clinodactyly and IUGR. Postnatal examinations of the boy were in a concordance with prenatal findings.
Even though the skeletal phenotype does not precisely match the SMC2, we believe the extension of the microdeletion during the parental transmission is likely causal.

PS04.71

Molecular analysis of 94 Short-Rib Polydactyly (SRP) individuals using targeted Next Generation Sequencing (NGS)

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Objective: The SRP group includes 4 lethal disorders (type I Saldino-Noonan, type II Majewski, type III Verma-Naumoff, type IV Beemer-Langer) 2 disorders compatible with life, asphyxiating thoracic dysplasia (ATD) and Ellis-van Creveld (EVC) syndrome and 2 related conditions, Sensenbrenner and Saldino-Mainzer syndromes. To date, 14 genes encoding primary cilia proteins have been involved in the SRP group. Here, we report the molecular screening of 94 SRP individuals by targeted NGS.
Methods: We performed targeted NGS of a customized ciliopathy gene panel, called ciliome, on a cohort of 94 individuals including 50 ATD, 23 EVC, 13 SPRIII, 2 SRPII, 1 SRPIV, and 5 Sensenbrenner.
Results: We identified 93 variations in 56/94 individuals. Among the 50 ATD cases, 30 were found to harbor variations in DYNC2H1 (21), IFT140 (2), IFT144 (2), WDR34 (1), WDR35 (1), WDR60 (1) or IFT43 (1), whereas and one heterozygote variation in DYNC2H1 and in IFT140 was identified in another case. DYNC2H1 variations were also identified in 9/13 SRPIII cases. WDR34 or IFT122 variations were identified in 2/5 Sensenbrenner cases. Finally, among the 23 EVC cases, EVC (9) or EVC2 (6) variations were identified in 15. Interestingly, a maternal deletion of EVC (4) or EVC2 (3) was present in 7 cases.
Conclusion: By ciliome analysis, we identified variations in ~60% of individuals. DYNC2H1 is responsible for ~48% of ATD-SRPIII spectrum, while EVC/EVC2 account for 65 % of EVC cases, with the presence of a maternal deletion in half cases. Exome sequencing is in progress for the remaining cases.

PM04.72

Bi-allelic TBX6 variations in a patient with spondylocostal dysostosis

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Background: Recurrent 16p11.2 microdeletions encompassing *TBX6* are associated with a wide-spectrum of symptoms including developmental delay, intellectual disability, autism spectrum disorder or obesity. Approximately 15% of patients with the recurrent deletion present abnormal segmentation of vertebrae (ASV). Recently, it was demonstrated that congenital scoliosis in 16p11.2 patients was associated with a specific *TBX6* haplotype in trans. Conversely, a unique family with autosomal dominant spondylo-costal dysostosis (SCD) secondary to a heterozygous *TBX6* stop-loss mutation. These observations suggest the existence of a continuous spectrum of ASV secondary to *TBX6* haploinsufficiency.

Methods: The CHU de Dijon is the french referring centre for SCD molecular diagnosis. The genes implicated in SCD, namely *DLL3, MESP2, LFNG, HES7* and *TBX6* were sequenced on a MiSeq instrument using Nextera XT protocol (Illumina). Array-CGH was performed by each genetic centre before molecular testing.
Results: A case with an inherited 16p11.2 microdeletion encompassing *TBX6* was referred because of a classical SCD. X-ray showed more than 10 contiguous ASV, associated with costal anomalies. Global examination was normal, with a normal psychomotor development, no visceral malformation. The deletion was inherited from an asymptomatic mother. The sequencing of the 5 genes identified a single *TBX6* missense (chr16:30100121G>T, CCDS10670.1:p.His221Asn) inherited from the healthy father. This genomic variation was absent from public databases, affected a conserved position (GERP: 6,02) and residue (PhastCons : 0,982).
Conclusion: Together with the literature data, this observation highlights the hypothesises of a continuous spectrum of ASV secondary to bi-allelic *TBX6* variations.

PS04.73
TGFB3-related connective tissue disorder : a third report with evidence of autosomal dominant inheritance

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Several new connective tissue disorders (CTD) recently came to attention. Among these, TGFB3 mutations were reported in two sporadic patients. We describe here a mother and her son with a heterozygous c.797G>A (p.Arg266His) missense variant in TGFB3. The index patient was evaluated for hypotonia and gross motor delay. When examined at 3 ½ y, epicanthic folds, anteverted nares, a long philtrum with full cheeks, a high-arched palate, a large uvula, joint hyperlaxity, scoliosis and marked pectus excavatum were noted. His mother had a history of recurrent joint dislocation. At 34 y, she presented with joint hyperlaxity, scoliosis, pectus carinatum, high-arched palate, a large uvula and a similar facial gestalt. Slit lamp examinations were normal. Maximal diameter of tubular aorta was 19 mm in the index case and 37 mm in his mother. This is the third report describing patients with a mutation in TGFB3. In 2013, Rienhoff et al identified a de novo missense mutation (c. 1226G>A; p.C409Y) in a young girl with hypotonia, low muscle mass, distal arthrogryposis, bifid uvula, hypertelorism, and hyperextensible large joints but no evidence of vascular disease. Matyas et al reported in 2014 a de novo missense mutation (c.899G>A; p.Arg300Gln) in a hypotonic girl with cleft soft palate, bifid uvula, hypertelorism, pectus excavatum, kyphoscoliosis, generalized hyperextensibility, aortic root diameter at the upper normal limit and mild mitral valve prolapse.
This new familial observation gives further support to the existence of a new autosomal dominant CTD comprising hypotonia, joint hyperlaxity, pectus carinatum/excavatum and a distinctive facial dysmorphism.

PS04.75
FLG gene mutations in urticaria development

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Urticaria is a kind of a skin rash that usually starts as an itchy patch and turns into swollen red welts. It is frequently caused by allergic reactions. Filaggrin is the major component of the stratum corneum and is involved in maintaining of skin barrier function. FLG gene loss-of-function mutations lead to epidermal barrier abnormality and are one of the main genetic risk factors for the allergic diseases development. Nevertheless, FLG gene mutations were not studied in urticaria before.
The aim of our study is the analysis of two FLG gene loss-of-function mutations (p.R501X and c.2282del4) in patients with urticaria and healthy individuals from Volga-Ural region of Russia. The patients group include 103 individuals of different ethnic origin (36 Russians, 30 Tatars, 32 Bashkirs and 5 individuals of mixed origin). The control group consists of 106 healthy donor without atopic diseases (24 Russians, 18 Tatars, 4 Bashkirs and 60 individuals of mixed origin). Mutations were genotyped by PCR-RFLP.
There are two individuals (1.9%) with urticaria in patients group who have c.2282del4 mutation. One of them has acute urticaria and another one has chronic from of the disease. The allelic frequency of the deletion is 0.96%. The allelic frequency of the c.2282del4 in healthy donors is 2.12%. Three patients (2.9%) with urticaria are heterozygous for the p.R501X mutation. Two of them have acute from of urticaria and one of them has chronic urticaria. The allelic frequency of the mutation in patients is 1.4% while in controls it is 0.53%. However all shown differences are not of statistical significance.

PM04.76
Type III collagen is important for collagen fibrillogenesis and for dermal and cardiovascular development

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Vascular Ehlers-Danlos Syndrome (vEDS) is a severe, life-threatening heritable connective tissue disorder, characterized by translucent skin, easy bruising, and propensity to rupture of arteries and hollow organs. The molecular basis of vEDS has been well-studied, showing a wide range of mutations in *COL3A1*, encoding type III procollagen. Most mutations lead to glycine substitutions in the helical domain of procollagen. Despite the identification of numerous *COL3A1* mutations, the mechanisms by which mutant type III collagen cause dermal and vascular fragility are not well understood, but factors, other than mechanical failure, are believed to contribute to the phenotype.
To study the role of type III collagen in development and disease, we generated a transgenic mouse model, which expresses *Col3a1* harbouring a typical glycine substitution (p.(Gly183Ser)) within the α1(III)-procollagen helical domain. *Col3a1*^{MUT} animals display clinical features reminiscent of human vEDS patients including thin translucent skin and wound healing problems leading to the development of severe transdermal skin wounds. Biomechanical testing revealed substantial fragility of the skin and aorta of *Col3a1*^{MUT} animals. Collagen fibrils in the skin and aorta were loosely packed, displayed a highly variable diameter, and secretion to the ECM was severely disturbed. The adventitia was significantly thinner and, smooth muscle cells made less connection with elastic fibers, and showed more intracellular space.
Together, our findings underscore a key role for type III collagen in collagen fibrillogenesis in skin and arterial tissue. This novel animal model provides opportunities for in-depth analyses of the role of abnormal type III collagen in vEDS, and for investigation of possible therapeutic interventions for this devastating disease.

PS04.77
Autosomal recessively inherited auto-inflammatory disorder (Weber Christian disease) responsive to tumour necrosis factor alpha inhibition

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Introduction: Weber Christian disease (also known as relapsing febrile nodular nonsuppurative panniculitis) is an eponymous condition characterised by idiopathic recurrent inflammation in the subcutaneous fat layer of the skin. The inflammation results in recurrent erythematous subcutaneous nodules with systemic symptoms including fever, myalgia, arthralgia, vomiting, and weight loss. The cause of this rare sporadic condition is unknown. Very rarely severe recurrent panniculitis has been reported in families with the PiZZ genotype of alpha 1-antitrypsin deficiency.
Clinical Summary: We describe a multiply consanguineous family in whom two siblings and a cousin have relapsing nodular panniculitis. This presented in the newborn period and continued as frequent flare-ups involving widespread painful lumps in the skin associated with fever, a raised CRP and white cell count. Additional features in this family include cataract, micronodular cirhosis, developmental delay and growth delay.
Initial treatment with systemic steroids and Anakinra partly ameliorated the condition. The cousin responded well to treatment with a monoclonal antibody against tumour necrosis factor alpha (TNF-α).
This family were recruited into the Molecular Pathology of Human Genetic disease study. Whole exome sequencing studies are in progress. We are looking for other similar families to join this study.
Conclusions: We report this very interesting family and ask for other families with a similar phenotype.
Grant and fellowships: Genetic Approaches to Novel Pathway Target Discovery in Human Genetic Disease with UCB Celltech.

PM04.78

A homozygous deletion of exon 1 in WISP3 causes progressive pseudorheumatoid dysplasia in 2 siblings

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Progressive pseudorheumatoid dysplasia (PPD) is a rare autosomal recessive disease, causing progressive joint stiffness, pain and typical radiographic abnormalities in young children. Classically, the disease presents with decreased joint mobility, in particular reduced hip movements, and a progressive involvement of metacarpophalangeal joints (MCPs), interphalangeal joints, wrists, elbows, knees, shoulders and ankles. The first symptoms mostly manifest in childhood, between three and eight years of age. There are no extra-skeletal manifestations. Stature of patients with PPD is normal in infancy but deviates generally to lower than the third percentile in adulthood. It is a progressive disease although the rate of progression is variable between patients.
Most patients are initially misdiagnosed because of the rarity of the disease and the unawareness of most clinicians. Presentation of PPD can mimic juvenile idiopathic arthritis as osseous joint swelling can be mistaken for synovitis. Although, there are no arguments for inflammation in PPD as inflammatory markers are always within the normal range. Radiographs can also help to distinguish, as destructive or erosive bone changes are never seen on radiographs from PPD patients.
PDD arthropathy is associated with loss of function mutations in the WISP3 gene, firstly described in 1999. Here, we describe the clinical case of two sisters suffering from PPD in whom molecular genetic analysis showed a homozygous deletion of exon 1 and the 5'UTR of the WISP3 gene. This is the first time that a gross deletion is described as the causal mutation in PPD.

PS05.01

Genetic variants in familial abdominal aortic aneurysms

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Introduction: Abdominal aortic aneurysm (AAA) is a frequent disorder with a prevalence of approximately 5% in the elderly population. In 20% of the patients AAA is familial. No genetic causes for AAA have been identified so far. The goal of this study is to identify genes that play a role in the formation of abdominal aneurysms.
Methods: The study includes approximately 950 AAA patients, 250 patients with- and 700 patients without a family history of AAA. So far we sequenced the DNA of 92 patients with a family history of abdominal aortic aneurysm. Whole genome sequencing (WGS) was performed in three families (15 individuals) and whole exome sequencing (WES) was performed in eleven families (29 individuals) and 48 single AAA patients with familial disease. Prioritization of resulting variants was performed according to the following gene sets:
1. Genes in diagnostics panel as applied in the Erasmus MC in thoracic or syndromic aneurysms (n=23)
2. A broad selection of genes involved in vascular function or disease (n=4209)
3. All genes in the genome
Results: We present the detailed workflow of the analysis of the genomics data, including the results so far. In six families a variant in one of the set 1 genes was found. Further analysis of the set 2 and 3 genes so far led to the identification of one candidate gene that shows variants in four AAA families and eight single familial AAA patients, and has not been linked to AAA before.
Supported by Stichting Lijf en Leven.

PM05.02

Plasma miRNA-208b and miRNA-499 as biomarkers of acute myocardial infarction

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Introduction: Acute myocardial infarction (AMI) is a leading cause of morbidity and mortality worldwide. Early and correct diagnosis might afford great benefits in treatment for AMI, thus the exploration of new biomarkers with high sensitivity and specificity in diagnosis of AMI is today a major goal. Recently, several studies indicated that microRNAs (miRNAs) circulating in serum or in plasma are attractive biomarkers for several human diseases. The objective of the present study was to examine the expression of the miRNAs miR-208b and miR-499 in plasma of AMI patients and to investigate whether these miRNAs could be useful biomarkers for AMI.
Materials and Methods: 20 AMI patients and 20 healthy controls were retrospectively recruited for a comparison of their plasma miR-208b and miR-499 expression. The levels of miRNAs in plasma were determined using TaqMan-based miRNA quantitative real-time polymerase chain reaction (qRT-PCR).
Results: Our data indicated that both miR-208b and miR-499 were significantly over expressed in plasma of AMI patients. We have found that the levels of miR-208b and miR-499 were increased by 16-fold and 128-fold, respectively, in plasma of AMI patients compared to healthy controls.
Conclusion: We conclude that plasma levels of miR-499 and miR-208b could be used as potential diagnostic biomarkers for AMI.

PS05.03

RNA expression profiling of Abdominal Aortic Aneurysmal disease

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Introduction: An abdominal aortic aneurysm (AAA) is a widening of the aorta below the renal arteries, usually asymptomatic until rupture causes fatal bleeding. AAA risk increases after the age of 65 years. Genetic susceptibility, smoking, hypertension, and atherosclerosis are established risk factors. Targeted ultrasound screening of high risk cases allows reduction of AAA related mortality, thus a better risk prediction model based on clinical characteristics and biomarkers is needed to earlier identify persons at risk. We aim to identify molecular markers for AAA disease by identifying genes involved in aortic vascular wall remodeling in AAA patients.
Methods: Microarray based genome wide expression analysis in affected aortic tissue of AAA patients was performed.
Results: In our gene expression studies in affected aortic tissues, we found genes which have been associated with AAA before: COL11A1, AdipoQ and LPL. Using Ingenuity IPA we identified immunity related pathways. Interestingly, expression levels of genes involved in the TGFβ signaling were significantly changed. We designed a flow chart to select genes as possible markers based on their level of expression, potential as blood marker and possible relevance for aneurysmal disease. We will validate these markers in experimental models for aneurysmal disease and study them further in blood of AAA patients.
Conclusions: Our analysis not only identifies genes and pathways previously associated with AAA genes, but also reveals novel genes and key regulators that will shed light on the processes involved in AAA formation and progression.
Funding: Euro Trans Bio and Stichting Lijf en Leven

PM05.04

Family screening for abdominal aorta aneurysm: don't forget the women

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Introduction: Abdominal aortic aneurysm (AAA) occurs in 6% of male and 1% of female population. Recommendations for family screening, allowing timely diagnosis and treatment, depend on estimates of risk for relatives. This study investigated risk for relatives and clinical risk profiles for familial AAA. Methods: Family histories of 568 AAA patients were classified as familial AAA when at least one first-degree relative was reported with an aortic aneurysm. Multivariable regression analysis was performed to identify clinical characteristics discriminating familial from sporadic AAA. Results: Familial AAA was reported in 23% of patients: 27% of female and 22% of male AAA patients. Female AAA patients had more affected relatives than male AAA patients (9.0% versus 5.9%, p = .022). Risk was increased 2 to 4 fold for relatives of male and female patients respectively. In familial AAA risk for relatives was increased 6 to 15 fold. Familial AAA was more frequent in young patients, without diabetes and hypertension.
Conclusions: Risk for relatives is much higher in familial
AAA than in the general population. When family history is not taken into account, an increase in risk remains and family screening is still recom-

mended. It is important that families of female AAA patients have similar access to screening as families of male patients, and that female relatives are screened.
The new insights in familial risk and family screening for AAA are important in counseling to inform AAA patients and allow their relatives to benefit from screening.
Funding: Stichting Lijf en Leven

PS05.05
Aortic dissection in a male with 1q21.1-q21.2 and 9p22.2 microduplications.

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We report a male patient who developed an aortic dissection at the age of 47. He is tall with the height of 191cm and weight of 83.5 kg, macrocephalic and normoteloric. He had mild joint hyperflexibility and a high arched palate. He did not meet diagnostic criteria for Marfan syndrome. He reported having developmental delay growing up and learning difficulties at school. He tested negative for an aortic aneurysm panel of 16 genes including *ACTA2, CBS, COL3A1, COL5A1, COL5A2, FBN1, FBN2, FLNA, MED12, MYH11, SKI, SLC2A10, SMAD3, TGFB2, TGFBR1* and *TGFBR2*.
An Affymetrix CytoScan HD microarray analysis showed an 1.935 Mb microduplication 1q21.1- q21.2 {arr[hg19] 1q21.1q21.2(145,895,746-147-,830,830)x3}, and another 0.951 Mb microduplication in 9p22.2 region {arr[hg19] 9p22.2(17,494,208-18,444,711)x3}. The 1q21.1-q21.2 microduplication was maternal in origin. The brother and the niece also had 1q21.1-q21.2 microduplication. The father was not available for genetic testing.
1q21.1 microduplication is associated with increased risk for developmental delay, autism spectrum disorder, schizophrenia, congenital heart disease, macrocephaly, tall stature and obesity. A recent study suggested that 1q21.1 duplication may cause abnormalities of connective tissue origin, such as cysts, varicose veins, carpal tunnel syndrome and congenital hip dysplasia. Significance of 9p22.2 duplication remains unknown.
To date aortic dissection has not been reported in patients with 1q21.1microduplication. We suggest that aortic dissection might be a possible complication of 1q21.1-q21.2 duplication and that patients carrying the duplication may need to undergo regular cardiac surveillance.

PM05.06
Absence of connective tissue remodeling in Smad3 mutant mice leads to aggressive and accelerated aneurysmal growth through disturbed downstream TGFβ signaling

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Aneurysm-osteoarthritis syndrome (AOS) is an autosomal dominant condition characterized by aortic aneurysms and early-onset osteoarthritis, caused by mutations in the SMAD3 gene. Smad3 is a part of the
key transcription factor complex Smad2/3/4, essential for TGFβ-activated downstream transcription of CTGF, MMPs, SMAD7 and others. $Smad3^{-/-}$ mice show aneurysmal development at young age, but the underlying mechanism is unknown.
Echocardiograms of cross-sectional studies in $Smad3^{-/-}$ mice showed a significant increase in diameter of root and ascending aorta (18-20%), and a significant increase in length (16-20%) already at age 6 weeks, but no difference in aortic distensibility. Importantly, 50% of $Smad3^{-/-}$ mice died suddenly between 6 and 24 weeks of age. Successive macroscopic analysis showed up to a 5-fold increase in ascending aortic diameter. We next performed longitudinal studies, showing a steep increase of aneurysmal growth within only 6 weeks. Since the aneurysmatic aortic wall remained translucent, this was indicative for the absence of large scale extracellular matrix remodeling or collagen deposition. Indeed, immunohistochemical analysis of $Smad3^{-/-}$ aortic walls showed
no increase in extracellular matrix accumulation, no excessive collagen or CTGF staining, nor loss of vascular smooth muscle cells (VSMCs).
$Smad3^{-/-}$ animals recapitulate the aortic phenotype observed in patients with SMAD3 mutations. Smad3 deficiency leads to imbalanced activation of downstream genes such as Smad6/7 and no activation of CTGF and MMPs in VSMCs. Initial minor dissections in the medial layers of the aortic wall trigger an extensive immune response and sudden acceleration of aneurysm formation, leading to rupture of
the aortic wall.

PS05.07
Lamin A/C gene mutations underlie Arrhythmogenic Cardiomyopathy with atrio-ventricular block

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Background: Arrhythmogenic Cardiomyopathy (AC) is an inherited heart muscle disease associated with mutations in genes mainly encoding desmosomal components, however mutations in extra-desmosomal genes can cause cardiomyopathies with overlapping AC features.
Case Presentation and Methods: A 76 years-old man diagnosed with biventricular AC died on the waiting list for cardiac transplantation. He had frequent syncopal episodes since young age, left bundle branch block and inverted T waves in V1-V3. He was implanted with pacemaker and then with cardioverter with frequent ventricular fibrillation shocks. Echocardiography exhibited a severe dilatation and hypokinesia in the right ventricle while the left ventricle was apparently normal. Genetic screening was performed by direct sequencing for all major desmosomal encoding genes on a Applied Biosystems ABI310 genetic sequencer and subsequently whole exome sequencing (WES) using TruSeq technology on a Illumina HiSeq2000 was carried out.
Results: Genetic screening was unable to detect genetic variations in desmosomal genes associated with the disease. WES identified a heterozygous point mutation c.949G>A, p.E317K in the lamin A/C gene (LMNA). This genetic variant, absent in the 1000 Genomes and Exome Variant Server datasets, has been previously associated with autosomal dominant Dilated Cardiomyopathy and atrio-ventricular block. The mutation was also detected in the proband`s daughter and son, who exhibited a AC-like pattern.
Conclusion: This study highlights that LMNA gene mutations can mimic the AC phenotype but always accompanied with atrio-ventricular block. A comprehensive genetic test enables a clear differential diagnosis in cardiomyopathies.

PM05.08
Co-inheritance of mutations associated with Arrhythmogenic and Hypertrophic Cardiomyopathy: how does it affect the phenotype?

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Background: Arrhythmogenic (ACM) and Hypertrophic cardiomyopathy (HCM) are genetically and phenotypically distinct diseases of the myocardium, showing an autosomal-dominant inheritance with incomplete penetrance and variable expressivity. They are the most common causes of sudden cardiac death in the young and athletes. Here we report 2 families with digenic inheritance of ACM and HCM mutations.
Methods and Results: The ACM probands of two families (A+B) were screened for mutations in 5 ACM genes (PKP2, DSP,DSG2, DSC2, JUP and CTNNA3). Moreover genetic analysis of the 2 major HCM genes (MYH7 and MYBPC3) was performed in 2 HCM patients, one for each family. In family A, 5 patients resulted to be double heterozygotes for a missense mutation (p.Met1601Ile) in DSP gene and a frameshift mutation (p.F305PfsX27) in MYBPC3 gene. The genetic analysis in Family B has identified the presence in 1 patient of an in frame deletion (p.del765L) in CTTNA3 gene and a missense mutation (p.Met877Ile) in MYH7 gene. Five single ACM and 7 single HCM mutation carriers were also found. Clinical evaluation of the 6 double heterozygotes has revealed a phenotypic heterogeneity with 1 asymptomatic case, 3 patients affected with ACM, 1 with HCM and 1 showing both phenotypes.
Conclusions: This is the first time that patients showing co-inheritance of ACM and HCM mutations are reported. The genotype-phenotype correlation in these rare patients indicates that such digenic inheritance is associated with heterogeneous phenotypes, probably due to the incomplete penetrance and variable expressivity of both mutations.

PS05.09
A founder PKP2 gene deletion in arrhythmogenic cardiomyopathy

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Background - Arrhythmogenic Cardiomyopathy (ACM) is an inherited heart muscle disease characterized clinically by ventricular arrhythmias and increased risk of sudden death, and pathologically by fibro-fatty replacement

of the myocardium. Conventional genetic testing of genes encoding desmosomal components results in a diagnostic yield of about 50% of ACM probands.
Aim - To search for large deletions/duplications in desmosomal genes in a large cohort of ACM index cases.
Methods and Results - Genetic screening for large deletions/duplications in 5 desmosomal genes was carried out in 68 unrelated index patients diagnosed affected with ACM according to revised 2010 Task Force criteria, and resulting negative for pathogenic point mutations in the same genes. Quantitative real-time PCR (qPCR) experiments were performed on an ABI PRISM 7900HT Sequence Detector by using at least three different sets of primer pairs located within exons from the proximal to the distal part of each gene. In 3 patients (4.4%) we identified a plakophilin-2 (PKP2) copy number reduction when compared to control samples thus suggesting a large heterozygous gene deletion. The PKP2 gene deletion was confirmed by Multiplex ligation-dependent probe amplification with the SALSA MLPA kit P168 (MRC-Holland). Haplotype analysis revealed a conserved haplotype among the PKP2 mutation carriers, strongly indicating a common founder.
Conclusions - Among desmosomal genes, only PKP2 shows large deletions of the entire coding region. These findings support the importance of expanding genetic testing in ACM patients with inclusion of PKP2 large deletion analysis when the results of conventional sequencing are negative.

PM05.10
A novel locus on chromosome 19p13.3 linked to arrhythmogenic cardiomyopathy

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BACKGROUND:
Arrhythmogenic cardiomyopathy (ACM) is an autosomal dominant myocardial disorder at risk of sudden death in the young and athletes. Thirteen causative genes have been found, with a central role of the desmosomal genes. Since causative mutations in ACM genes have been detected in about 50% of probands, additional disease genes remain to be identified.
METHODS and RESULTS:
In a large ACM family, where the proband resulted negative for mutation screening of desmosomal genes, genome-wide linkage analysis highlighted a shared region of 2 Mb on chromosome 19p13.3 (multipoint LOD score=3.85). CNV analysis was carried out in order to exclude the presence of structural variations. Whole exome sequencing was performed in 4 affected patients, through two different platforms (HiSeq2000 Illumina, Ion Torrent) at a mean coverage of 80X. Sequencing data didn't reveal the presence of any novel variant shared by the 4 subjects, neither into the linkage region nor in the rest of the exome. Exons with insufficient reads (≤15 depth) of the 13 ACM genes and of the genes inside the critical region were further evaluated by Sanger sequencing but no additional coding mutations were found. Only a novel intronic variant (c.766+8C>A) in TMEM43 gene was identified. The segregation of this variant among all the available family members excludes an association with the disease phenotype.
CONCLUSION:
In this ACM family showing no mutations in known ACM genes segregating with the disease phenotype, a novel locus was mapped on chromosome 19p13.3 and a critical region of 2 Mb was defined.

PS05.11
Inducible pluripotent stem cell technology as a tool to study Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC) in a Maritime family

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ARVC is an inheritable disease featuring potentially lethal arrhymias and heart failure characterized pathologically by fibrofatty replacement of cardiaomyocytes (CMs). We have ascertained a large Maritime family segregating a form of ARVC that is mutation-negative for all genes currently known to cause ARVC, suggesting that a novel variant must be responsible. Using DNA derived from formalin fixed, paraffin embedded tissue, as well as from blood or saliva of known carriers and relatives, we have performed extensive genetic investigation to identify the novel mutation responsible for the disease in this family (i.e. SNP genotyping, haplotype analysis, whole-exome and whole-genome sequencing). We have identified candidate genomic regions shared among affected individuals, however the precise causative mutation remains elusive. To assist in identifying the causative factor and to study the underlying disease mechanisms, we have used a novel inducible pluripotent stem cell (iPSC) technique to generate disease specific CMs that were derived from B-lymphocytes of ARVC patients. Human iPSC lines were successfully developed by using episomal plasmid vectors to over express reprogramming factors. CMs were then differentiated from iPSC by modulating Wnt/β-catenin signaling. Currently, gene expression analyses with RNAseq from RNA isolated from iPSC derived CMs that were generated from affected and healthy control samples are underway. The iPSC technique used in this project may help identify the specific mutation that causes this specific form of ARVC, enabling life-saving intervention in at-risk individuals. Additionally, this technique offers a potential pathway for identifying causal variation in diseases in which coding-sequence changes are not obvious.

PM05.12
Bioinformatics analysis of the genomic candidate markers for diagnostic panel of atherosclerosis

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Despite the significant achievements in the field of medicine and biology, morbidity and mortality from atherosclerotic lesions remain at a high level, and the range of adequate antiatherosclerotic therapy goals is still limited due to the lack of validated biomarkers. Therefore, the main goal of our research is the development of a new reliable genomic indicators panel for early, pre-clinical diagnostics of possible future disease.
We performed a bioinformatics analysis of potential candidate genes directly or indirectly involved in the pathogenesis of atherosclerosis. The analyzed genes list included lipid metabolism, matrix metalloproteinases, folate cycle, oxidative stress genes and some others. The motifs search was implemented using bioinformatics package MEME Suite.
In the investigated genes vicinity we detected 670 motifs, homologous pre-mi-RNA, and 4300 motifs, homologous mature mi-RNA. The average mi-RNA distribution density in genomic sites ranged from 0 to 2.2 pre-mi-RNA per 1000 p.n. and mature mi-RNA - from 0 to 6.9 per 1000 p.n. Inside the investigated genes we identified 433 motifs, homologous pre-mi-RNA, and 2780 motifs, homologous mature mi-RNA. The average motifs distribution density amounted to 0.4 per 1000 p.n. for pre-mi-RNA and 3.3 per 1000 p.n. for mature mi-RNA. The most frequently encountered were motifs, homologous mmu-mir-466i, hsa-mir-5096, hsa-mir-1273g, ppy-mir-1268 and hsa-mir-619. According to the MirTarBase data we established that hsa-miR-138-5p is the multigene regulator for the studied genes groups.
The obtained results could be used to develop atherosclerosis diagnostic panel.
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PS05.13
Whole exome sequencing identifies a novel germline mutation in calcium ion channel; associated with atrial fibrillation.

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Introduction - AF is the most prevalent sustained cardiac arrhythmia, responsible for considerable morbidity and mortality. Due to the complex pathophysiology of AF the underlying mechanisms are incompletely understood. In the few cases of familiar form of AF, where the causative mutation has been found, it has been identified in genes encoding ion-channels involved in sodium or potassium handling. Nevertheless in the majority of cases with familiar forms of AF the cause is still unknown.
Methods and Results - Whole exome sequencing was performed on seven individuals from a Danish family, presumably with a highly penetrable monogenetic form of AF. Quality control showed that the whole exome could be investigated sufficiently.
Analysis, following Broad institute current best practices, revealed 2 novel variants (not found in over 60,000 controls, of which 2000 Danish). Interestingly one of the novel mutations was found in a calcium ion channel. In silico predictions supported the pathogenic potential of this variant, predicted damaging in 7/8 prediction tools.
Discussion - In this study a calcium ion channel variant that co-segregated

fully with AF in a large family was identified as the potential monogenetic cause of AF. This study suggests a previously undescribed pathophysiological pathway for AF.

PM05.14

Next-generation sequencing of AVNRT patients identifies high prevalence of mutations in genes involved in sodium and calcium handling

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Atrioventricular nodal re-entry tachycardia (AVNRT) is the most common paroxysmal supraventricular tachycardia, affecting women twice as frequently as men, often before turning 40 years. Familial clustering, early onset of symptoms, and lack of structural anomaly indicate involvement of genetic factors in the development of AVNRT. However, the field is poorly investigated, and a great amount of work is to be done, in order to reveal the plausible genetic causes.
We hypothesized that AVNRT patients have a high prevalence of mutations in 38 genes potentially involved in arrhythmic diseases and highly expressed in the heart.
Next-generation sequencing of 38 genes previously associated with cardiac arrhythmia and highly expressed in the atrioventricular conduction axis was applied to the DNA profile of 99 AVNRT patients using Haloplex target enrichment system and the results were verified using an Illumina next-generation sequencing amplicon-based platform, the TruSeq Custom Amplicon Kit.
In total, we found 28 mutations in 11 genes; 25 missense mutations, two synonymous, and one in-frame mutation. Twelve of these were novel. The main part of these mutations are in genes responsible for sodium and calcium handling, such as SCN5A, SCN10A, the CACNA genes, ATP2A2 encoding the SERCA pump, and RYR2, indicating that AVNRT might be a sodium- and calcium-channel disease like Brugada Syndrome as opposed to atrial fibrillation which in addition has a potassium channel affection. This is, to our knowledge, the first study to report a genetic component in AVNRT.

PS05.15

The first SCN5A founder mutation in a Belgian cohort of Brugada syndrome patients

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Brugada syndrome (BrS) is a heritable primary electrical disorder with typical ECG alterations accounting for up to 20% of sudden cardiac death in young patients (<45 years) without apparent structural cardiac abnormalities. BrS is genetically heterogeneous and so far a dozen causative genes have been identified, of which SCN5A is the predominant gene. At present a causal mutation is identified in about 20-30% of BrS patients. SCN5A encodes a sodium channel and loss of function mutations will lead to a decreased sodium inward current. In the Netherlands, an SCN5A founder mutation (p.1795insAsp) was previously described, which gives rise to an overlap syndrome between BrS, Long QT syndrome and cardiac conduction defects (Postema et al., 2009).
Here, we present 23 BrS patients of 17 different Belgian families carrying the identical c.4813+6_4813+7insGGGT mutation in SCN5A. This mutation, previously described in two families of Western European descent, creates a cryptic splice site in exon 27 leading to complete loss of function of the sodium channel (Hong et al., 2005; Rossenbacker et al., 2005). We identified a shared haplotype consisting of seven genetic markers spanning a region of 5.2 Mb. Based on the haplotype size, we estimate that the common ancestor of these families lived approximately 300 years ago. The clinical spectrum of mutation carrying individuals ranges from asymptomatic to full blown BrS. In this cohort, a relatively low number of sudden cardiac death has been observed.
As such, we have identified the first Belgian BrS founder mutation. These families will be instrumental for the future identification of BrS phenotype modifying genes.

PM05.16

Molecular autopsy of sudden cardiac death with structurally normal heart: the circumstances of death and ECG tracings can address the final diagnosis

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Background. Sudden cardiac death with normal heart can occur in the setting of inherited ion channel diseases. Among these, Brugada syndrome (BrS) is characterized by non-ischemic ST segment elevation associated with PR prolongation and right bundle branch block, whereas Lenégre disease is characterized by atrio-ventricular block with progressive structural changes of the conduction system. In these arrhythmic syndromes, more than 1300 nucleotide variations has been identified in SCN5A gene.
Methods & Results. A 35-years old asymptomatic male died suddenly during sleep. Post-mortem examination excluded extracardiac causes of sudden death as well as the absence of structural abnormalities in the working myocardium. Personal clinical history re-assessment revealed a 'coved-type' ST segment elevation on right precordial leads with PQ prolongation (220 msec) in a patient's ECG performed during blood donation, compatible with BrS. Detailed conduction system investigation by serial section technique showed severe fibrosis of the bifurcating His and proximal bundles with sclerotic interruption of the left bundle branch. Genetic screening of SCN5A (NM. 198056.2) gene identified a mutation in exon 22 (c.3673 G>A , E1225K), defined as "likely to be pathogenic" by in silico tools, and previously linked to BrS phenotype with atrio-ventricular block. Cascade genetic screening detected 5 additional family mutation carriers, and provoked electrical stimulation unmasked ECG abnormalities in 2 of them.
Conclusion. Lenégre disease and BrS are overlapping clinical entities, «bookends» in a continuum of sodium current deficiency, accounting for a structural cardiomyopathy of the specialized conducting tissue occurring in the setting of an otherwise normal heart.

PS05.17

Functional analysis of a coding variant in ZC3HC1 at 7q32.3 associated with protection against coronary artery disease

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Genetic and environmental factors are believed to contribute equally to the risk of coronary artery disease (CAD). The CARDIoGRAM Consortium first identified a coding variant, rs11556924, (MAF, 0.38) in the *ZC3HC1* gene associated with protection against CAD ($p= 9.8x10^{-18}$; OR= 0.90). The amino acid change (Arg363His) is in a conserved region of the gene and is predicted to have functional effects (Polyphen-2). The gene product of *ZC3HC1*, Nuclear Interaction Partner of ALK (NIPA), is a regulator of cellular proliferation and is an essential component of an SCF-type E3 ligase complex. This complex catalyses the ubiquitination of proteins (such as cyclin B1) targeting them for proteasomal degradation. We have investigated the functional effects of this amino acid change in *ZC3HC1*. Here we demonstrate a) allele specific differences in mRNA expression in whole blood, b) differential ERK dependent phosphorylation of Arg363 vs His363, c) a small but reproducible decrease in mobility of the risk variant, Arg363 by Fluorescence Recovery After Photobleaching (FRAP), that may reflect differences in their steady state interaction with nuclear partners. Additional studies are underway to further characterize effects on NIPA structure and activity, relevant to cardioprotection.
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PM05.18

Is lipoma-preferred partner (LPP) a cause of cardiac malformations with reduced penetrance?

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There are two published reports of variants in LPP (OMIM #600700) in association with isolated conotruncal defects and congenital heart disease in VACTERL association. Variants have included deletions and missense mutations. Here we report a child with transposition of the great arteries (TGA) and coarctation of the aorta (CoA) with a deletion of multiple exons of LPP. We discuss the evidence for the role of LPP in the causation of cardiac malformations.
The 2 ½-year-old patient was born at 37 weeks gestation. TGA and CoA were diagnosed on day 1 and surgically corrected shortly after. Developmental milestones were normal and the child and her mother have no dysmorphic

features. Array CGH (BlueGnome Cytochip oligo ISCA 4080-5 8x60k array) identified a 205kb interstitial deletion involving exons 2-4 of LPP (#600700) in the child. The deletion was inherited from her unaffected mother.
LPP has been linked to cell-cell adhesion, cell motility and transcriptional regulation however a mechanistic link between LPP function and cardiac embryogenesis has not yet been established. We have performed a comprehensive review of mutation databases (DECIPHER, ISCA and DGV) for other LPP deletions and identified 21 patients and 9 controls with deletions varying from large (12.6 Mb) deletions to small exonic and intronic (5.2 Kb) deletions. Three of these individuals were reported to have cardiac malformations.
Our case provides an additional case of LPP deletion associated with congenital heart disease raising the possibility that rare loss-of-function variants of LPP may be associated with cardiac, in particular, conotruncal malformations, with reduced penetrance.

PS05.19

A microRNA target site in *SHOX2* associates with cardiac arrhythmia

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Cardiac arrhythmias contribute significantly to cardiovascular morbidity and mortality. Developing approaches towards disease prediction and treatment of heart rhythm disorders is therefore an important topic in healthy aging. Although environmental factors play an important role in the pathogenesis of cardiac arrhythmias, multiple studies have demonstrated that there is also a strong genetic component. Molecular pathways involving the homeodomain transcription factor Shox2 control the development and function of the primary cardiac pacemaker. Genotyping untranslated regions of the *SHOX2* gene in 378 arrhythmia patients and 1870 control individuals demonstrated a significant association between a 3'UTR variant and the disease (p=0.00515, OR=2.373). Patients with the 3´UTR variant clinically presented significantly longer PR intervals. Mechanistically, this variant creates a novel functional microRNA binding site. Circulating microRNA levels in plasma were found to be significantly altered in all testable patients carrying the 3'UTR variant, compared to patients with the wild type allele (p=0.0095).
These results provide direct evidence that a genetic variant in the *SHOX2* gene is associated with cardiac arrhythmias and point to a microRNA as potential biomarker and therapeutic target in this disease.
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PM05.20

Genetic Analysis of Cardiac Arrhythmias in Patients from Saudi Arabia

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Hereditary arrhythmias are hereditary due to mutations in genes responsible for normal cardiac rhythm generation and propagation. These genes include KCNQ1, KCNH2, SCN5A, RYR2, CASQ2. Mutation detection leads to proper clinical diagnosis, therapy, behavioral management, and family screening. This may decrease the incidence of sudden cardiac death.
Our center took a pioneering initiative to set up a comprehensive cardiac arrhythmia genetic research and diagnostic laboratory to provide specialized cardiogenetic clinical services in Saudi Arabia and neighboring countries and to create a database of local mutations to develop a "genomic chip" to be used in routine screening for cardiac arrhythmia patients in these countries. Our ultimate goal is to discover new genes in arrhythmia pathology, which will lead to development of novel gene targeted pharmacotherapy for cardiac arrhythmia. Presently, we receive referrals from all over the Kingdom and neighboring countries.
Over 3 years, 46 families were assessed, and genetic analysis was performed in our lab. Arrhythmogenic mutations were found in 29 index patients (63%). Cascade screening of family members identified 176 relatives with mutations, 80% which were founder mutations. Some were more common in specific geographic regions. All mutation carriers had variability in clinical presentation of the arrhythmias (no symptoms to sudden death). In 6 families, index patients presented with arrhythmias and deafness, (Jervell Lange and Nielsen syndrome JLNs), with mutations in both alleles of the KCNQ1 gene. The proportion of JLNS among LQTs was higher than most population studies. We identified novel, recurrent and also founder mutations in the genes involved in cardiac function.

PS05.21

Genetic investigation of exons of 100 heart genes in a forensic setting

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Introduction: Sudden cardiac death (SCD) is responsible for a large proportion of sudden, unexpected deaths in young individuals (<50 years). In forensics, one-third of the cases remain unexplained after autopsy. In the remaining two-thirds, structural cardiac abnormalities are found, although often unspecific and without a clear cause of death. Sudden deaths are frequently suspected to be caused by inherited cardiac diseases. Implementation of genetic investigations in forensic medicine may increase the diagnostic rate in cases of sudden death in the young. The purpose of the study was to explore the utility of genetic testing using next-generation sequencing (NGS) by investigating the frequency of pathogenic variants in 100 genes associated with cardiac diseases in a cohort of suspected SCD victims. Methods: Genetic investigation of 133 unrelated, young SCD victims with (1) sudden unexplained death (SUD), or (2) non-diagnostic structural cardiac abnormalities without cause of death, or (3) structural cardiac abnormalities diagnosed as cardiomyopathy, was performed. Using NGS, all exons and UTR regions of 100 genes associated with inherited cardiomyopathies and cardiac channelopathies were investigated. Results: The results show that 45% of SUD cases, 35% of cases with non-diagnostic structural cardiac abnormalities and 48% of cases with cardiomyopathy had likely pathogenic variants in one or more disease-related genes. Conclusion: Screening by NGS of these 100 genes revealed that between one-third and half of the suspected SCD victims had likely pathogenic genetic variants. Large-scale genetic testing seems a powerful supplementary tool in forensic investigations in sudden death cases and increases the diagnostic efficiency.

PM05.22

Study of the consequences of *BAG3* molecular variants associated with human dilated cardiomyopathy

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Introduction: Dilated cardiomyopathy (DCM) is a rare pathology defined by left ventricular dilatation and reduced ejection fraction, leading to heart failure and often heart transplantation. We identified DCM-causing dominant heterozygous mutations in *BAG3* (*BCL2-associated athanogene 3*). BAG3 is a cytoprotective co-chaperone involved in protein homeostasis, and plays a role in clearance of proteotoxic misfolded, aggregated, or altered proteins. Deleterious *BAG3* mutations are located in the BAG domain, which interacts with the chaperone Hsp70 family (heat shock protein 70). We hypothesized that *BAG3* mutations induce cardiomyocyte dysfunction due to impaired cellular proteostasis.
Methods/Results: We studied biochemical and cellular effects of BAG domain mutations in heterologous cell models with transient overexpression of GFP-BAG3 chimeric proteins, or in the zebrafish model organism. Rat neonatal cardiomyocytes overexpressing $BAG3^{mut}$ displayed disorganized sarcomeres, BAG3-positive cytoplasmic aggregates, and atrophy, resulting in cell death. Using GST pull-down and co-immunoprecipitation, we demonstrated a loss of $BAG3^{mut}$-Hsp70 interaction, indicating strong structural

effects of *BAG3* mutations and potential impairment of HSP70-dependent proteostasis. Accordingly, using heat-shock induced transient denaturation of luciferase reporter mimicking proteotoxic stress, we showed decreased protein refolding in HEK cells transfected with $BAG3^{mut}$ compared to $BAG3^{wt}$. Finally, zebrafish microinjected with mutant cDNA developed pericardial edema mimicking cardiac dysfunction.
Conclusion: Our results strongly suggest that *BAG3* mutants fail to bind to HSP70, blunting HSP70-dependent response to proteotoxic stress. Such functional alteration leads to protein aggregation, cardiomyocyte loss and probably cardiac insufficiency. It reveals a new putative DCM pathological mechanism.
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PS05.23
TTN truncating mutations are a major cause of dilated and noncompaction cardiomyopathy

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Cardiomyopathies are characterized by extreme genetic and clinical heterogeneity, with >50 causative genes known to date. With Sanger sequencing, in about 45% of the hypertrophic cardiomyopathy (HCM) patients a pathogenic mutation was identified, whereas only 15-20% of the dilated cardiomyopathy (DCM) and noncompaction cardiomyopathy (NCCM) cases were solved. Nowadays we make use of a targeted NGS panel (48 genes) including the Titin gene (*TTN*), the largest known human protein that plays a central role in sarcomere organization. *TTN* truncating mutations have been found to be a major cause of DCM, but these type of mutations have not frequently been reported in NCCM or HCM.
Sixty-seven NCCM patients, 84 DCM patients and 150 HCM patients were tested with a NGS targeted sequencing approach, including the largest isoform of TTN (NM_001267550.1).Twelve DCM patients (~14%) and 9 NCCM patients (~13%) showed a truncating mutation in exons encoding the A-band of TTN (exons 259-359), whereas no mutations were detected in HCM patients in this region. This A-band of Titin was recently reported to contain the majority of truncating mutations in DCM patients (Roberts *et al.* Sci Transl Med. 2015,14;7; 270).
In conclusion, targeted NGS is an effective approach for cardiomyopathy diagnostics, resulting in increased diagnostic yield for all types of cardiomyopathies. For DCM patients, the majority of mutations are caused by truncating mutations in *TTN*. We now report that truncating mutations in *TTN* also are a major cause of NCCM.

PM05.24
Whole exome sequencing (WES) in 41 Czech families with inherited cardiovascular diseases

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WES facilitates genetic diagnostics of inherited cardiovascular diseases, enables genetic stratification and may eventually foster individualized therapies. Due to integrated cardio-genetic care, altogether 96 families with ≥ 2 affected individuals were characterized and WES was carried out in a total of 41/96 families (TruSight Exome, Illumina, USA). 23/41 families suffered from dilated cardiomyopathy (DCM), 1/41 - non-compaction cardiomyopathy (LVNC), 14/41 - hypertrophic cardiomyopathy (HCM), 1/41 -arythmogenic cardiomyopathy (ACM) and 2/41 had unexplained cardiac arrest (UCA). Detected variants were confirmed by Sanger DNA sequencing and by segregation analysis. WES revealed a putative molecular genetic mechanism in 22/41 (64%) families. The pathogenic mutations were identified in 12/22 (54 %) DCM, 1/1 LVNC, 13/14 (92%) HCM, 1/1 ACM and in 1/2 familes with UCA. The most frequently mutated gene in DCM was TTN (23%) and in HCM the MYH 7 (29%). In case of LVNC, the likely pathogenic variant was identified in the OBSC, for ACM in PKP2 and in the family with UAC in SCN5A genes, respectively. Although disease genes were already identified, most mutations were novel. Our results and mutation distribution are in accordance with other studies. Our model of integrated genetic care of patients with inherited cardiovascular diseases is the first one in Czech Republic. Supported by: FNM 00064203, CZ.2.16/3.1.00/24022, NF-CZ11-PDP-3-003-2014, LD14073, IGA NT13770.to M.M. and IKEM 00023001 to V.M.

PS05.25
Functional analysis of the 9p21 coronary artery disease-associated locus using isogenic cell lines generated by genome editing

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Introduction: Genome-wide association studies (GWAS) have identified multiple loci associated with coronary artery disease, the most significant of which is located on chromosome 9p21.
Harismendy et al (Nature, 2011) suggest that the causal variants at this locus are two SNPs that disrupt STAT1 transcription factor binding to an enhancer element. This may therefore result in altered gene expression of nearby protein and RNA genes. Here, we investigated this hypothesis by utilising genome editing to produce isogenic cell lines that differ at only the two SNPs of interest. This allows any functional effects of these variants, in response to inflammatory signalling, to be determined.
Methods: Genome editing by recombinant adeno-associated virus (rAAV) was used to specifically alter the genotype of the two SNPs in HeLa-S3 cells, with all other variants remaining constant. Heterozygous lines were derived from the homozygous-protective parental line.
Genotype-specific variation in STAT1 binding was assessed following interferon-γ treatment using chromatin immunoprecipitation and quantitative PCR (ChIP-qPCR). Local gene expression of treated cells was also investigated using qPCR.
Results: ChIP-qPCR revealed a reduction in STAT1 binding by approximately 50% in cells heterozygous for the risk alleles compared to the homozygous non-risk control. However, no difference in gene expression of CDKN2A, CDKN2B and several ANRIL transcripts was observed. Analysis of additional local genes is on-going.
Discussion: The observed reduction in STAT1 binding suggests that these SNPs are functional, but the absence of a gene expression difference indicates that other functional variants in the region may be required to contribute to disease.

PM05.26
A novel de novo TFAP2B mutation in a sporadic patient with Char syndrome

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Char syndrome first described by Florence Char in 1978, is a rare autosomal dominant disorder characterized by patent ductus arteriosus (PDA), facial dysmorphism, and anomalies of the fifth finger. Char syndrome is caused by mutations in TFAP2B gene encoding a neural crest-derived transcription factorthat play an important role in development, apoptosis, cell-cycle control, and complex morphogenic processes. Also it is suggested that TFAP2B mutations may be associated with isolated nonsydromic PDA. Here we described a 4 year-old male patient associated with PDA, clinodactyly of the fifth fingers, facial dysmorphism and developmental delay. Sequence analysis of TFAP2B gene revealed a single-base heterozygousc.698T>A (cDNA.867T>A, V233D) mutation in exon 4 which results in the replacement of valine by aspartic acid at codon 233. Parental mutation screening detected wild type genotype. To the best of our knowledge, this is the first report of V233D mutation in TFAP2B gene associated with Char syndrome.

PS05.27
High yield of copy number variations in individuals with complex congenital heart disease in Saudi Arabs

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Introduction
Congenital heart diseases (CHDs) are complex cardiac disorders that manifest in different forms, such as tetralogy of Fallot (TOF), ventricular septal defects (VSDs), atrial septal defects (ASDs), coarctation, and may occur in combination with other defects. Copy number variations (CNVs) have been implicated in many complex disorders, including cardiovascular and cancer. However, the impact of such variants on congenital heart disease (CHD) manifestation is not fully explained yet.
Methods
In the present study, we selected and performed Copy Number Analysis on 50 Saudi (Arab) cases of complex CHDs, including TOFs, bicuspid aortic val-

ve (BCV), tricuspid atresia (TCA) and subaortic membrane (SAM) disorders, to determine the possible link of the disease to changes in copy numbers.

Results

Among the 50 individuals, 22 exhibited CNVs on four chromosomal regions. Out of these were 16 TOFs exhibiting duplications, three of which were linked to chromosome 17q21.31, two to 16p11.2, and one each to 2q13,, 19p13.3 and 20p12.3. Furthermore, one TOF together with three complex cases involving BAV, and SAM, Kartagener's syndrome, exhibited deletions at 16p11.2 and 15q11.2. Interestingly, an individual case with TCA showed copy number gain at 2q13, while another with Kartagener syndrome showed a copy number loss at the same locus. These duplications/deletions involved sequences of 420-1200 kbp. The qPCR verification assays on five of the studied individuals confirmed the duplications and deletions found by cytoscanning.

Conclusion

Our study reveals a high yield of CNVs in CHD, mostly in the regions of chromosomes 16p11.2 and 17q21.31, which could be used clinically to identify such patients routinely.

PM05.28

Sporadic congenital heart disease and copy number gains on chromosome 21

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Congenital heart disease (CHD) is 1000 times more common in people with Down syndrome (DS) than the general population. Complete atrioventricular septal defects have the strongest association with DS, and the combination of AVSDs and tetralogy of Fallot is almost unique to people with DS. To evaluate the hypothesis that copy number (CN) gains on chromosome 21 are associated with sporadic CHD we analysed more than 2000 non-DS case samples and 800 controls using the Illumina 660W quad array. After an extended quality control pipeline, CN gains larger than 10kb called by both QuantiSNP and Penn CNV algorithms were included. Cases were enriched for copy number gains in DSCAM and COL6A1 which have been previously identified as candidates for DS-related CHD. These results suggest they may also have a role in sporadic, non-DS CHD.

PS05.29

Polygenic risk for coronary artery disease is associated with cognitive ability in older adults

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Introduction: Coronary artery disease (CAD) is associated with cognitive decrements and risk of later dementia, but it is not known if shared genetic factors underlie this association. We tested whether polygenic risk for CAD was associated with cognitive ability in community-dwelling cohorts of middle-aged and older adults.

Materials and Methods: Individuals from Generation Scotland: Scottish Family Health Study (GS:SFHS, N=9865) and from the Lothian Birth Cohorts of 1921 (LBC1921, N=517) and 1936 (LBC1936, N=1005) provided cognitive data and genome-wide genotype data. Polygenic risk profile scores for CAD were calculated for all of the cohorts using the largest available GWAS data set, the CARDIoGRAM consortium (22,233 cases and 64,762 controls). Polygenic risk profile scores for CAD were then tested for their association with cognitive abilities in the presence and absence of manifest cardiovascular disease.

Results: A meta-analysis of all three cohorts supported a negative association between CAD polygenic risk and fluid cognitive ability, verbal intelligence and memory. Whereas these findings were not statistically significant in the Lothian Birth Cohorts, in GS:SFHS polygenic risk for CAD was negatively and significantly associated with fluid cognitive ability (β = -0.02, p = 0.03), Mill Hill Vocabulary test (β = -0.03, p = 0.006) and Logical Memory (β = -0.02, p = 0.042).

Conclusions: Increased polygenic risk for CAD is associated with lower cognitive ability in older adults. Common genetic variants may underlie some of the association between age-related cognitive decrements and the risk for CAD.

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PM05.30

A loss-of-function mutation in the haptoglobin gene is associated with a decrease in serum haptoglobin and an increase in non-HDL cholesterol and cardiovascular risk

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Human haptoglobin is a plasma protein which binds free hemoglobin released by the destruction of red blood cells, thereby protecting against oxidation. Whether serum levels of haptoglobin contribute to cardiovascular disease remains controversial. Here, through whole-genome sequencing of 2,230 Icelanders followed by imputation into 5,411 Icelanders with serum haptoglobin measurements, we found a rare (minor allele frequency = 0.57%) loss-of-function mutation associated with a decrease in serum haptoglobin levels (effect = -1.28 SD per minor allele, $P = 7.4x10^{-25}$). In addition, this variant associated with an increase in non-HDL cholesterol (effect = 0.18 SD per minor allele, corresponding to 0.18 mmol/l, $P = 1.2x10^{-5}$) and increased risk of coronary artery disease (OR = 1.26, $P = 0.011$). This mutation is a splice donor variant in the haptoglobin gene (*HP*) on chromosome 16q22 and represents a founder mutation in the Icelandic population. This signal was independent of a common copy-number variation in the *HP* gene and a previously reported common SNP (rs2000999) at that locus, previously shown to associate with haptoglobin levels. In addition to haptoglobin, these common variants were also associated with non-HDL cholesterol, demonstrating an inverse relationship between their effects on the two traits.

In summary, we report a loss-of-function mutation in the *HP* gene, present in about 1 out of 90 Icelanders, which is associated with a decrease in serum haptoglobin levels and an increase in non-HDL cholesterol and cardiovascular risk. Our genetic results point to a role of haptoglobin in cholesterol metabolism.

PS05.31

Identification of rare potential CAD-causing variants in GWAS loci

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The identification of genes causing coronary artery disease (CAD) is one of the most important tasks to understand the disease and to develop potential new treatments. Genome-wide association studies (GWAS) have identified more than 50 loci associated with CAD. If common variants increases the risk of CAD, it is likely that rare variants within the same locus also increase the risk. Indeed, the presence of such variants may even point the GWAS locus to the causal gene. Further, as rare variants are expected to have stronger effects, unraveling the disease mechanism might be easier. Hence, this study aims to identify rare variants in known CAD risk genes and to find potential causal genes in CAD GWAS loci.

We screened for variants in a total of 82 genes close to or harboring CAD associated SNPs ($p<5x10^{-8}$). For these candidate genes, we screened for rare (<1%) truncating variants in a pool of 255 exome sequenced early onset MI patients with a positive family history. Each variant was validated by Sanger sequencing and tested for co-segregation with CAD in respective family members.

We only identified a small number of rare variants in a subset of the candidate CAD genes. Exemplary, LDLR and ABCG8 are known to harbor rare variants increasing CAD risk and we confirm these findings. In addition, we also report rare variants in genes where only common risk variants are reported so far. For instance, to our knowledge, we are the first to report rare loss-of-function variants in *NBEAL1*.

PM05.32

A Mesenchymal Stem Cell-Based Approach for Investigating Cardiovascular Disease-Related Genetic Variants

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Introduction: Large-scale genetic studies have identified many loci that contribute to complex disease. We have explored the use of Mesenchymal Stem Cells (MSCs) as a system for creating genotype-specific cell models to investigate the biological mechanisms of complex disease-related genetic variants in vitro. Here we describe the differentiation of MSCs down a vascular

smooth muscle (VSMC) lineage in order to investigate a coronary artery disease (CAD)-associated genetic variant.
Materials and Methods: MSCs were isolated from umbilical cords using an explant method. Flow cytometry and StemPro® Differentiation Kits were used to characterise MSCs. MSCs were differentiated to VSMCs using TGF-β1, and characterised by qPCR, immunofluorescence and calcium imaging.
Results: A bank of 114 umbilical cord-derived MSCs was created, and characterised by cell surface markers (99.7% positive for CD105, CD90 and CD73, and 0.2% positive for the negative markers CD45, CD34, CD11b, CD19 and HLA-DR) and differentiation into adipocytes, chondrocytes and osteoblasts. MSCs were differentiated towards a VSMC lineage, demonstrated by an upregulation of VSMC markers; ACTA2, TAGLN2 and CNN1 ($p<0.001$). As a proof of concept, we investigated the functional CAD-associated coding variant, rs3825807, located in the protease ADAMTS7 in MSCs carrying either the risk or non-risk allele. A trend showed MSCs with the risk allele had increased migratory ability, consistent with findings reported by Pu et al., (2013). We are currently undergoing studies with differentiated VSMCs.
Conclusion: Our findings show that our MSC bank can be used to study genetic variants associated with complex disease including CAD-related phenotypes.

PS05.33

Mypn knock-in mutant mice develop dilated cardiomyopathy

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Dilated cardiomyopathy (DCM) causes severe heart failure, but even with over 30 causative genes identified, DCM pathophysiology is poorly understood. In addition, current treatments only ameliorate symptoms without treating underlying causes, therefore developing genetic therapies is essential. We identified human DCM mutations in *MYPN* (myopalladin), and created a knock-in mouse model carrying one such mutation. We also introduced two synonymous variations into the murine recombination vector, creating an experimentally validated shRNA target sequence permitting specific extinction of mutant allele expression. Introduced variations did not interfere with normal allelic splicing, nor allelic expression ratios in either skeletal or cardiac muscles when assessed via allele-specific qPCR. Mice were fertile, with normal growth rates and long-term survival. Mutant heart gross morphology appeared normal, but electron micrographs demonstrated thickened sarcomeric Z-lines. Echocardiography until 18 months demonstrated that both heterozygote and homozygote mutant mice displayed cardiac insufficiency from the age of three months. In mutant mice, systolic ventricular diameter and volume were increased, and ejection fractions and fractional shortening reduced, confirming systolic dysfunction. In contrast to most published murine models of dominant human DCM which display recessive phenotypes, we generated a knock-in mouse model of persistent mild dominant DCM, highlighting its particular utility for potential allele-specific genetic therapy. Future analysis of mutant cardiomyocyte contractile properties and calcium handling will provide further insights into dominant *Mypn* DCM genotype/phenotype pathophysiological relationships.
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PM05.34

Next generation sequencing for the molecular genetic characterization of patients with dilated cardiomyopathy

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Dilated cardiomyopathy (DCM) is an important cause of heart failure and the leading indication for cardiac transplantation worldwide. Genetic etiology plays an important role in disease pathogenesis. The aim of our study was genetic testing of 49 patients with diagnosed DCM using next generation sequencing technologies (SOLiD 5500xl). All probands underwent a clinical examination. High blood pressure, coronary disease, chronic excess of alcohol consumption and systemic disease were excluded prior to genetic analysis. Genomic DNA was extracted from peripheral blood leukocytes or buccal swab sampling according to standard protocols. Molecular-genetic analyses included 50 different genes implicated in causing DCM. In the survey of selected Slovak patients with dilated cardiomyopathy missense mutations in GM2A, DHX57, NDUFV2, COL12A1, EXD1, IBA57, LAD1, MORN2, KRT15 genes were detected. In 33% of cases missense mutation - pathogenic mutation rs1805124 T/C in the gene SCN5A channel (responsible for the reduction of PR and QRS intervals) including mutations in the intron regions: rs7428779 C/T and rs41312433, G/T were identified. In five patients was found the rare variant rs11408120, -/G in TCEAL6 gene. The variant was confirmed by Sanger sequence analysis using ABi 3500 xl DNA Sequencer (Applied Biosystems). In recent years the progress in identifying the genetic causes of cardiomyopathies. Introduction of screening methods forms potential benefits of gene based diagnosis to identify individuals at risk for developing heart failure.
This study is the result of implementation of the projects APVV-0644-12 and ITMS 26220120241.

PS05.35

Genomic study of familial and idiopathic dilated cardiomyopathy in Mexican patients.

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Dilated cardiomyopathy (DCM) is a disease of the myocardium caracterized by left ventricular dilatation, systolic dysfunction and myocardial fibrosis. It is a major cause of morbility and mortality and the first indication for cardiac transplantation. Clinical manifestations are variable and include heart failure, thromboembolism and sudden cardiac death. The etiology may be genetic for up to 50% of cases either sporadic or familial (F-DCM). Most F-DCM cases have an autosomal dominant form of inheritance (90%). More than 40 genes have been associated with the disease, and the frequency of mutations varies from one population to another. The purpose of our study is to describe the mutations producing DCM in Mexican patients. Coding exons and intron flanks of 31 well characterized genes responsible for DCM, were sequenced by NGS with the Ion Torrent technology looking for pathogenic genetic variants in Mexican patients diagnosed as having idiopathic or F-DCM. Preliminary results in 22 index cases show mutations in the *TNNT2, RBM20* and *CSRP3* genes, and two different mutations already detected in patients with Brugada Syndrome, in the *SCN5A* gene. In one index case we found a mutation in the *MYSH7* gene associated to Hypertrophic cardiomyopathy (HCM). Also, two new SNVs causing a stop gain, one in TTN, the other in LMNA were found in different patients. At present, we detected a known mutation in six patients and a new variant in two. We are confirming by capillary sequenciation and completing the study including the available relatives.

PM05.36

Truncating titin mutations cause a mild and treatable form of dilated cardiomyopathy

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Background
Truncating mutations in Titin (TTN) occur in 25% of dilated cardiomyopathy (DCM) cases, but also in 3% of healthy controls. We studied whether truncating TTN mutations cause a clinically distinguishable form of DCM .
Methods
We compared clinical data of probands and their relatives with either a truncating TTN mutation or a pathogenic mutation in Lamin A/C (LMNA), to probands with DCM who tested negative for both.
Results
We studied 240 subjects: TTN probands (n=47) and their relatives (n=73), LMNA probands (n=31) and their relatives (n=29), and probands negative for both (TTN/LMNAneg DCM; n=60). Median follow-up was at least 2.5 years in each group. TTN mutation carriers presented at a significantly higher age with DCM than LMNA mutation carriers (48.6 vs 41.5 years, $p=0.045$ for probands; 59.7 vs 47.0 years, Log-Rank $p=0.01$ for relatives). TTN mutation carriers less often developed left ventricular ejection fraction (LVEF) below 35% (HR=0.50, $p=0.02$ for probands), and had better outcome (less composite endpoints (HR=0.10, $p<0.001$ for probands; HR=0.21, $p=0.02$ for relatives) compared to LMNA mutation carriers, and compared to TTN/LMNAneg DCM patients (HR=0.33, $p=0.05$)). Strikingly, after initiation of standard heart failure treatment an LVEF increase of at least 10% occurred in 50.0% of the TTN subjects, while this only occurred in 6.3% of LMNA subjects ($p<0.001$) and 22.2% of TTN/LMNAneg DCM subjects ($p=0.03$).
Conclusions

This study shows that truncating mutations in TTN induce a DCM that is less severe at presentation and more amenable to standard therapy than either LMNA mutation induced DCM or TTN/LMNAneg DCM.

PS05.37

Assessment of the mechanical stability of the aorta in a mouse model of Ehlers-Danlos syndrome vascular type (EDS IV)

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Introduction: EDS IV is an autosomal dominant connective tissue disorder caused by mutations in the COL3A1 gene and associated with an increased risk for aortic rupture. So far, only disease management and treatment of symptoms are available. Haploinsufficient mice of a recently described novel mouse model of Col3a1 show reduced mechanical stability of the aorta and spontaneous death (~28% of cases) due to aortic rupture similar to the human EDS IV phenotype. Our goal was to characterize the mechanical stability of the aorta in this mouse model compared to wild-type mice.
Methods: 1.5-mm-long sections of aortic arch as well as ascending and descending aorta from Col3a1 mice were mounted on two 200-µm diameter stainless steel wires on a TissuePuller (Danish Myo Technology) and stretched radially until tissue damage, thereby continuously recording the stretching force (in mN).
Results: Maximum force at tissue damage was significantly lower in heterozygous Col3a1 mice compared to age- and gender-matched wild-type animals in both the ascending and descending parts of the aorta. For both genotypes, the mechanical stability of the aorta was decreasing, with increasing distance from the heart.
Conclusions: We developed a protocol for the assessment of the mechanical stability of mice aorta, which is suitable to detect significant differences between heterozygous and wild-type Col3a1 mice. Our results open the way to test pharmacological substances for their potential to increase the mechanical stability of the aorta with the goal to find a targeted therapy for patients with EDS IV and related aortic disorders.

PM05.38

The type of variants at the COL3A1 gene associates with the phenotype and severity of vascular Ehlers-Danlos syndrome

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Vascular Ehlers-Danlos syndrome (vEDS) is a rare and severe autosomal dominant disorder caused by COL3A1 gene variants, with little evidence of genotype-phenotype correlation. Clinical characteristics and course of disease of 215 molecularly-proven patients (146 index-cases and 69 relatives), were analysed. We found 126 distincts variants that were divided into five groups : 1) Glycine substitutions (71 variants in 127 patients), 2) splice-site and in-frame insertions-deletions (36 variants in 55 patients), 3) variants leading to haploinsufficiency (7 variants in 14 patients), 4) non glycine missense variants within the triple helix (4 variants in 7 patients), 5) non glycine missense variants or in-frame insertions-deletions, in the N- or C-terminal part of the protein (8 variants in 12 patients). Overall, our cohort confirmed the severity of the disease with a median age at first complication of 29 years [IQR 22 to 39], the most frequent being arterial (48%) and digestive (24%) ruptures. Groups 2 and 1 were significantly more severe than groups 3-5 with extreme median ages at first major complication of 23 to 47 years. Patients of groups 3-5 had a less typical phenotype and remarkably absence of digestive events. The glycine-replacing amino-acids were frequently destabilizing residues of the collagen assembly. Thus, the natural course of vEDS and the clinical phenotype of patients are influenced by the type of COL3A1 variant. This study also confirms that patients with variants located in the C- and N-termini or leading to haploinsufficiency have milder course of the disease, and less prevalent diagnostic criteria. These findings may help refine diagnostic strategy, genetic counselling and clinical care.

PS05.39

Differential DNA methylation in individuals with a history of cardiovascular diseases

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Cardiovascular disease (CVD) is among the leading causes of death worldwide. There are several known genetic and lifestyle risk factors, but association between epigenetics and CVD is poorly understood. In this study, we investigate the link between DNA methylation and CVD. We performed an epigenome-wide association study in a population-based cohort (N=730). Participants were not ascertained upon disease background, but some had a history of CVD (Table). DNA methylation was measured at 470789 autosomal CpG-sites. Differentially methylated sites were identified for a subset of diseases (Table). Some sites were located in genes previously associated to CVD, e.g. BNIP3 and GDF15. Enrichment analysis identified a number of molecular functions and biological processes, which are overrepresented among the differentially methylated genes. For example, participants with a history of myocardial infarction had an enrichment (FDR q-value=0.00142, Enrichment = 4.69) of differential methylation in genes associated with cardiac muscle tissue growth. The identified genes are good candidates for additional studies to further understand the genesis and progression of CVD, as well as for the development of therapies and treatments.

Table

CVD	No. affected individuals (%males)	No. significant CpG (FDR q-value < 0.05)
Cardiac Arrhythmia	5 (60%)	1
High Blood Pressure	147 (40%)	0
Myocardial Infarction	48 (58%)	211
Stroke	27 (56%)	0
Thrombosis	22 (59%)	0

PS05.41

Exome sequencing reveals novel functional mutation in APOB causing Familial Hypercholesterolaemia

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Introduction: APOB mutations are a rare cause of Familial Hypercholesterolaemia (FH) and routine genetic diagnosis only includes the study of two small APOB fragments (exon 26 and 29). Recently functional mutations have been described in APOB fragments not routinely studied and our group characterized 2/5 as causing FH.
The main aim of this project was to identify and characterize novel alterations in APOB in order to identify the genetic cause of the hypercholesterolemia in these patients.
Methods: we performed whole-exome sequencing of 5 Portuguese clinical FH patients apparently mutation negative. All results found in APOB were analysed. For functional studies LDL from index patients and relatives was separated and marked with FITC-LDL for studies by flow cytometry in HepG2 and U937 growth assays.
Results: We identified 2 alterations in exons 19 and 26 in 2 patients. In vitro analysis of exon 26 alteration (p.Thr3826Met) carrier showed a decrease in binding and internalization of LDL and in U937 growth assays, showing a similar effect as APO3527. As reported before, also in this family the penetrance is also reduced. Alteration in exon 19 had a neutral effect.
Conclusions: The spectrum of functional alterations in APOB outside the fragments routinely screened is growing. Screening of all 29 exons of APOB should be performed in routine diagnosis, now possible by NGS. It is expected that a further 10% of clinical FH patients can have FH due to a novel APOB mutation. On this basis we are currently sequencing a panel of 95 negative patients.

PM05.42

LDLR functional in vitro assays: a step forward for the correct genetic diagnosis of familial hypercholesterolaemia

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Aim: Familial hypercholesterolaemia (FH) is an autosomal dominant disorder that confers an increased cardiovascular risk, due to high levels of cholesterol in blood since birth. FH has a prevalence of 1:500, being estimated that >90% of these patients have mutations in the *LDLR*. Although more than 1600 alterations have been identified, the majority of them remain without functional studies.
The aim of this work is to construct vectors for the *in vitro* study of common alterations in Portuguese FH patients, contributing for phenotype/genotype clarification.
Methods: The 14 most common *LDLR* alterations to date without functional assessment were selected. *In silico* assessment was performed using described tools. Site-directed mutagenesis was performed in a pcDNA3_LDLR plasmid and functional assessment is being performed in CHO-ldlA7 cells by flow cytometry. Complementarily, protein expression and immunofluorescence studies will be performed.
Results: *In silico* prediction classified 7/14 alterations as benign (p.Met1Leu, p.Gly20Arg, p.Gln92Glu, p.Asp221Tyr, p.Ile473Val, Ala606Ser and p.Ile764Thr) and 7 as probably pathogenic (p.Cys184Tyr, p.Gly207_Ser213del, p.His211Asp, p.Glu626Lys, p.Glu288Lys, p.Asp601Val, p.His656Asn). 9/14 alterations were also reported in several other countries as mutation causing disease. Among these constructs, 11 of the mutagenesis occurred successfully and the functional assessment is underway. The remaining are being performed.
Conclusions: Although more than 1600 variants have been described in the *LDLR*, there are still novel variants being found, proving its heterogeneity. It is imperative to functionally characterize these alterations to contribute for the elucidation of the molecular basis of FH worldwide. An accurate diagnosis and early personalized counseling/treatment, will improve FH patients prognosis.

PS05.43

Familial Hypercholesterolaemia - An efficient genetic testing laboratory service for multiple lipid centres using Next Generation Sequencing and PASS

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NICE recommends comprehensive genetic testing in all patients clinically diagnosed with FH, and genetic cascade testing of at-risk relatives. The Cardiovascular Disease Outcomes Strategy (2013) has improving case ascertainment for FH (12 to 50%) as a key target. British Heart Foundation are supporting implementation of a cascade testing programme for England through start-up funding bids, however, the cost of FH genetic testing can still remain a barrier to commissioning.
High throughput automated NGS genetic testing processes large numbers of samples at reduced cost using a unique patient sequence barcode identifier. The custom-designed targeted capture assay (HaloPlex, Agilent) sequences 4 FH genes; *LDLR*, *PCSK9*, *APOB* and *LDLRAP1* and the *SLCO1B1* variants (*rs2306283* and *rs4149056*) associated with statin-induced myopathy. Data analysis uses an open-source pipeline; alignment (bwa), variant calling (GATK), variant annotation (Geneticist Assistant, SoftGenetics), and copy number analysis (CONTRA/bespoke CNV tool).
Bristol Genetics Laboratory (BGL) has tested >600 FH index cases by NGS from >15 referral centres with a 35% positive detection rate, the most common mutations being *APOB* c.10580G>A and *LDLR* c.313+1G>A. CNV analysis has detected 12 *LDLR* deletions and 1 duplication. 7% of patients have variants of uncertain significance (VUS) with the majority of these found in *APOB*. 116 relatives have undergone cascade testing.
BGL is trialing the online PASS database to link the genetic testing process directly to lipid clinics using workflow management tools. We report the genetic test results on our cohort, and our service developments, highlighting the efficiencies of using an NGS testing approach and PASS.

PM05.44

Identification of familial hypercholesterolemia in patients with early onset MI by systematic molecular genetic analysis

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Familial hypercholesterolemia (FH) is an oligogenic disorder characterized by markedly elevated low-density-lipoprotein (LDL)-cholesterol levels. Despite the knowledge of the FH candidate genes, it is largely under-diagnosed in European countries. Since FH increases the risk for coronary artery disease (CAD) and myocardial infarction (MI), it might be specifically overlooked in the large number of such patients.
Here, we systematically examined the frequency of potential FH-causing variants in LDLR, APOB, PCSK9 and STAP1 by exome sequencing in 255 German patients with premature MI and a positive family history for CAD. We further performed co-segregation analyses in an average of 5.5 family members per MI patient.
In total, we identified eleven potential disease-causing variants that co-segregate within the families. Eight variants were previously reported as disease-causing and three are novel (LDLR.p.V271I, PCSK9.p.D204N and STAP1.p.T47A). However, exome sequencing also revealed that some variants, which have been reported to cause FH, do not co-segregate with the disease in respective families.
The data reveals that 1 out of 20 patients with premature MI carries potential FH-causing mutations. Hence a large proportion of FH patients escapes the diagnosis. Systematic molecular-genetic screening for FH may assist and unravel the diagnosis in a substantial number of cases and thereby allow a timely intensive or preventive treatment of FH. Interestingly, some of the previously reported disease-causing variants could not be validated in this study. Our results highlight the need for careful evaluation of each potential disease-causing variant prior to reporting.
This Study was supported by the Deutsche Forschungsgemeinschaft cluster of excellence ‚Inflammation at Interfaces'.

PS05.45

Anomalous retinal vasculature in MYH11-associated Familial Thoracic Aortic Aneurysms and Dissection (FTAAD)

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Background: Familial thoracic aortic aneurysms and dissection (FTAAD) is a heterogeneous autosomal dominant condition which compromises the wall of the thoracic aorta, and in some cases other vessels. MYH11 mutations have been reported in families with FTAAD associated with patent ductus arteriosus (PDA).
Case report: We here report a three-generation family with a splice site mutation in intron 27 of MYH11. The proband was a 29-year-old woman who died 11 days post-partum due to two separate areas of dissection in the thoracic and abdominal aorta. On family screening, her 12-year-old son was found to have a PDA, and her 56-year-old father was found to have dilatation of the proximal ascending aorta (4.1cm), and a subsequent CT angiogram identified tortuosity of the innominate artery. No clinical features suggestive of a wider disorder of connective tissue were found in the proband's father or son, but both shared the MYH11 splice mutation. On ophthalmological examination, the proband's father also had anomalous vascular loops identified in both eyes.
Conclusions: The family reported here exhibits the range of phenotypes associated with FTAAD caused by MYH11. In general, there is little genotype-phenotype correlation in FTAAD, but the presence of PDA directs suspicion towards MYH11. In addition, one family member had retinal vascular tortuosity, possibly resulting from his generalised abnormality of the vasculature. The prevalence of retinal vascular anomalies in FTAAD requires further investigation; if widely prevalent, ophthalmological examination would provide a valuable non-invasive test of extra-cardiac vascular status.

PM05.46

FBN1 gene polymorphysms association with dilatative pathology of the ascending thoracic aorta (DPATA)

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In most cases the causes leading to the aortic wall expansion and/or dissection remains unclear. Certainly, this pathology is caused by aortitis, atherosclerosis or inherited as a single gene mutation. Evidence has shown that FBN1 mutations may predispose DPATA in the absence of phenotypic cha-

racteristics of Marfan syndrome and lately FBN1 polymorphisms drew more attention to cause sporadic DPATA. Based on LeMaire et al study in 2011, we investigated polymorphisms of the FBN1 gene (rs2118181, rs1036477, rs10519177, rs755251, rs4774517) in a case-controlled study for DPATA from Lithuanians.
We studied 312 patients who had undergone aortic reconstructive surgery for DPATA. Patients were subdivided into test groups according to the DPATA phenotypes: ascending aortic aneurysms, dissectionsandpost-stenotic-dilatation. The control group (n=472) was obtained from a random sample screened within epidemiological studies of the Lithuanian population. The FBN1 polymorphisms were investigated by real-time polymerase-chain-reaction amplification. Fisher's exact test, $\chi 2$ test and allelic association odds ratio were used for statistics. The distribution of genotypes was conformable with Hardy-Weinberg equilibrium ($p>0.15$).
We observed that minor alleles of all five FBN1 SNPs were significantly associated with aortic dissection with OR 2.13-2.59, $p<0.001$, and two SNPs: rs2118181 and rs1036477- with an increased risk of ascending aortic aneurysm with OR 1.67, CI 95% 1.61-2.40.There were no significant associations between all studied FBN1 SNPs and post-stenotic aortic dilatation.
Minor alleles of all SNPs investigated might be considered as risk alleles for aortic dissection and two of them (rs2118181, rs1036477) are increasing odds for aortic aneurysm formation.

PM05.48

Upregulation of Antigen Presentation Pathway under the treatment with neuropropeptide Semax in a rat model of brain focal ischemia

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Neuroprotective synthetic oligopeptide Semax composed of a fragment of ACTH4-7 and C-terminal tripeptide Pro-Gly-Pro is used for therapy of acute stroke. The molecular mechanisms of its neuroprotective action have been hitherto unknown. The response of the transcriptome of ischemized rat brain cortex tissues to the action of Semax in the male Wistar rat brains was investigated. The intraperitoneal injection of peptide was done at 15 min, 1, 4 and 8h after permanent middle cerebral artery occlusion (pMCAO). mRNA expression change was analyzed in 24h groups versus 3h groups following pMCAO and "pMCAO + Semax". The Illumina RatRef-12 Expression BeadChip was used in our study. The action of Semax enhanced the expression of 17 genes (Ap1, B2m, Cd74, Hla-C, Hla-Dma, Hla-Dmb, Hla-Dqa1, Hla-Dra, Hla-Drb1, Hla-E, Psmb8, Psmb9, Tapbp) belonged to the major histocompatibility complex (MHC). We identified significant overlap between 13 of these genes and the Antigen Presentation Pathway ($p=4.4E-11$). BioRank Ingenuity ranking system assigns to this pathway the high scores that means that this set of changed expression genes hit a critical component of the pathway in the Ingenuity Canonical Signaling Pathway library. Besides we observed changed levels of transcripts of 7 genes (Cd74, Hla-A, Hla-Dra, Psmb8, Psmb9, Tap1, Tapbp) belonged to the MHC in the "ischemia" group during one day after pMCAO. But for the Antigen Presentation Pathway this set of genes was scored lower than in the case of neuropeptide treatment. Our data assume that Semax affects the immune response during the active stage of ischemia.

PS05.47

A human laterality disorder associated with a homozygous WDR16 deletion

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Visceral asymmetry is determined through embryonic ciliary motion. A failure to generate the normal left-right (L-R) asymmetry during early stages of embryogenesis may result in severe anatomical abnormalities, including heterotaxy syndrome (HS) which consists of abnormal L-R axis arrangement of the abdominal and thoracic viscera and situs inversus totalis (SIT) which manifests by mirror image asymmetry of the internal viscera. HS is at times accompanied by complex congenital cardiovascular anomalies whereas SIT is frequently associated with Primary Ciliary Dyskinesia (PCD). The genetic etiology of defects not associated with PCD is largely unknown.
In this study we investigated the cause of situs anomalies, including HS and SIT (PCD excluded), in a consanguineous family.
Whole exome analysis including thorough coverage analysis, revealed a homozygous deleterious deletion in the WDR16 gene - chr17. hg19:g.9481617_9489649del8033.
The finding was confirmed both by cDNA analysis and by the results of Multiplex Ligation-dependent Probe Amplification analysis which confirmed segregation of the deletion in the family. Serial PCR reactions using intronic primer sets, resulted in the amplification of a genomic fragment which contained the breakpoint.
WDR16 protein was previously proposed to play a role in cilia-related signal transduction processes; the rat Wdr16 protein was shown to be confined to cilia possessing tissues and severe hydrocephalus was observed in the wdr16 gene knockdown zebrafish.
The phenotype associated with the homozygous deletion in our patients suggests a role for WDR16 in human laterality patterning. Exome analysis is a valuable tool for molecular investigation even in cases of large deletions.

PS05.49

Hereditary hemorrhagic telangiectasia (Rendu-Osler-Weber syndrome): first results of molecular genetic testing in the Czech republic

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Hereditary hemorrhagic telangiectasia (HHT) is an autosomal dominant disorder characterized by an aberrant vascular development. HHT affects approximately 1 in 5000 people and is caused by pathogenic variants in a number of genes involved in the TGF-β signaling pathway. Endoglin (ENG) and activin receptor-like kinase-1 (ALK1/ACVRL1) encode proteins expressed on vascular endothelial cells. These genes are casually related to HHT. The clinical diagnosis of HHT is based on recurrent epistaxis due to telangiectases and arteriovenous malformations (AVMs) which can occur in the pulmonary, cerebral and hepatic circulation leading to stroke, internal hemorrhage, and severe anaemia. We used classical sequencing approaches to perform molecular characterization in 18 clinically affected unrelated probands with the suspected diagnosis of HHT, and detected a total of 7 different mutations in the two genes. Two mutations were identified in the ENG gene, both deletions, one of which was novel, in exon 8 and exon 11. There were tested also several family members of these probands and we identified 5 and 2 mutation carriers, respectively. Three of five mutations identified in the ALK1/ACVRL1 gene were novel and comprised missense mutation in exon 6, deletion in exon 3 and small indel in exon 8. No mutations were found in ENG/ACVRL1 in 11 probands. Genetic testing can confirm the clinical diagnosis in individuals and identify presymptomatic mutation carriers. Once a famililal mutation is identified, relatives at risk can be tested. Individuals with a mutation are identified for intensive clinical surveillance. We offer genetic counseling for at-risk relatives.

PM05.50

Sarcomeric gene mutations in Turkish families with hypertrophic cardiomyopathy

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Background: There is little knowledge about familial hypertrophic cardiomyopathy in Turkey. In this study, our aim was to determine a causing mutation in three sarcomeric genes (MYH7, MYBPC3 and TNNT2) in Turkish families with HCM and high-risk for sudden cardiac death (SCD).
Materials and methods: The study included twelve index cases of early onset (<40 years) clinically diagnosed HCM patients with a positive family history for HCM and SCD. All participants were evaluated with a detailed history, physical examination, 12-lead electrocardiography and two-dimensional echocardiography. DNA was extracted from peripheral blood and coding regions and flanking intronic sequences of MYH7, MYBPC3 and TNNT2 genes were screened using array-based re-sequencing. All novel variants and known mutations were confirmed with Sanger sequencing. After the causal mutation in family members was screened.
Results: From 12 index cases, a known missense mutation was found in 6 individuals and also novel missense mutation was found in 2 individuals. Four different causal mutations in the cardiac-beta myosin heavy chain (MYH7) gene, one in the cardiac myosin binding protein C (MYBPC3) gene and one in the cardiac troponin T (TNNT2) were found. In addition, two novel intronic variants in MYH7 and MYBPC3 genes were found in 2 different index cases. Detection of the novel missense mutations and intronic variants within the family members and control population is ongoing.
Conclusion: Our preliminary result provides a general view of the familial

hypertrophic cardiomyopathy with high-risk for SCD and highlights the importance of mutation screening for these three genes in Turkey.

PS05.51
Disease causing variation is differentially distributed in MYH7 but not in MYBPC3 in patients with hypertrophic cardiomyopathy

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Hypertrophic cardiomyopathy (HCM) is an inherited disorder of the heart muscle. HCM has been linked to dominant genetic variants in sarcomere genes, most commonly in *MYH7* and *MYBPC3*. The *MYH7* and *MYBPC3* gene products interact but have distinctly different functions: *MYH7* encodes the myosin head, the molecular motor that binds and pulls actin, while *MYBPC3* is a regulatory protein that binds to both the myosin and actin. Despite these functional differences, variants in both genes lead to a similar presentation of HCM. To investigate the differences between the variant profiles of *MYH7* and *MYBPC3*, we combine published and unpublished data from three centers (Stanford University, the Mayo Clinic, Harvard University). The combined dataset included results from 4349 patients. In *MYH7*, 215 out of 234 (91.9%) unique damaging variants are missense, while only 164 out of 343 (47.8%) are missense in *MYBPC3* (Fisher $p=1.25x10^{-6}$). We compared variant locations in *MYH7* and *MYBPC3* from HCM patients with those from 60,706 individuals in the Exome Aggregation Consortium (ExAC). We find a significant difference in the distribution of HCM and ExAC variant locations in *MYH7* (KS $p=3.95x10^{-13}$), but not in *MYBPC3* (KS p=0.462). The peak of differential variant density in *MYH7* covers the head, the lever arm, and the beginning of the tail of the myosin heavy chain molecule. Our results suggest differing regional biophysical contributions to the pathogenicity of disease for *MYH7* and *MYBPC3*.

PM05.52
Cardiac Ankyrin Repeat Protein (CARP/Ankrd1) gene variants in Hypertrophic Cardiomyopathy

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CARP/Ankrd1 belongs to the muscle ankyrin repeat protein (MARP) family involved in a mechano-signaling pathway that links myofibrillar stress response to muscle gene expression. Also, CARP/Ankrd1 has an important role in transcriptional regulation, myofibrillar assembly, cardiogenesis and myogenesis. Few studies supported a role of CARP gene variants in the etiology of hypertrophic (HCM) and dilated cardiomyopathy. We have performed screening for mutations/variants in Ankrd1 coding sequences in 50 familiar or idiopathic HCM patients. Two missense heterozygous CARP variants in exon 2 (P52A and R66Q) were identified, each in one patient. Preliminary functional analysis of these variants on protein level were performed in rat neonatal cardiomyocytes by Fluorescent Recovery After Photobleaching (FRAP) assay. The results of the FRAP experiments showed difference in mobility between wt and P52A variant, while R66Q exerted similar behavior as wt protein. Our findings point to possible disruption of the protein-protein interaction and disturbance of the normal cardiac signaling in cardiomyocytes caused by P52A allele variant of CARP/Ankrd1.
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PS05.53
Investigation of Pathogenic Genes in Chinese Hypertrophic Cardiomyopathy Patients by Whole Exome Sequencing

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Hypertrophic cardiomyopathy (HCM) is a cardiovascular disease with high heterogeneity. The limited knowledge about ~40% patients suggests that it needs to be investigated to understand the pathogenesis of the disease. A large number of variants were identified by whole exome sequencing in seventy-four HCM patients passing Sanger method sequencing eight HCM causative genes. After filtering against multiple databases and functional filter, 3228 SNPs and 475 InDels in 3046 genes were identified. TADA model was then applied using exome sequencing data of 2000 controls and 74 cases and 99 genes gained priority, with DNAH11, OBSCN ranking the first and second place, respectively. Associated analytical tools as DAVID and IntPath were also adopted to explore the novel mechanism underlying the HCM phenotype. The results showed that various genes and gene sets related to cytoskeleton, ATPase activity, dynein and calcium transport played critical roles in the pathogenesis of HCM, with a lot of other processes as well, implicating many processes involved in the HCM phenotype. We also found two novel OBSCN variants that co-segregated with the HCM patients in two HCM families. Additionally, we described the OBSCN role in HCM. Our study provides a way for exploring the pathogenesis of a less understood HCM cohort.

PM05.54
Mutational spectrum of Filamin C (FLNC) in hypertrophic cardiomyopathy

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Exome sequencing identified the Filamin C (*FLNC*) as a hypertrophic cardiomyopathy (HCM) candidate gene.[1] The *FLNC* putative mutations segregated with the disease in several families. We also demonstrated that patients with *FLNC* mutations showed marked sarcomeric abnormalities in cardiac muscle, and functional studies revealed that these *FLNC* variants resulted in the formation of large filamin C aggregates. Our aim was to characterize the mutational spectrum of *FLNC* in a large cohort of HCM patients. We performed a Next-generation sequencing of *FLNC* in 335 HCM patients, including 117 sarcomeric-mutation carriers. We identified a total of 25 HCM index cases who were heterozygous carriers of putative mutations, 21 in the 218 patients without sarcomere-mutation (10%) and 4 in the 117 sarcomere-positive cases (3%; $p<0.05$). Only five of the 25 *FLNC* variants were reported in healthy subjects in the Exome Sequencing Project (ESP) database, but all of them at a frequency <0.001%. Three variants, p.A1247V, p.A1539T, and p.A2340V, were found in two index cases. In nine of the 14 families we had several affected members, and all were mutation carriers. Interestingly, some of the *FLNC* mutations were linked to sudden cardiac death episodes. In conclusion, our work suggested that *FLNC* mutations were a significant cause of HCM.

[1]Valdés-Mas R *et al*. Mutationsin filamin C cause a new form of familial hypertrophic cardiomyopathy. Nature communications. 2014;5:5326).

PS05.55
The Clinical Utility of a Proton-Ion Ampliseq-based Cardiac Gene Panel

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Introduction: Whole exome sequencing (WES) and whole genome sequencing (WGS) are increasingly being used in clinical practice. However, these approaches continue to be expensive, time consuming and difficult to interpret. So far, clinical WES studies have yielded diagnostic results in ~ 25% of cases. Hence, a complementary easier, equally sensitive method needs to be developed.
Method: A 406 cardiac related (cardiomyopathy, congenital heart disease, arrhythmias, vascular aneurysms) gene panel (with 10490 amplicons) based on Ion Torrent AmpliSeq technology was developed. So far, 243 gDNA samples were sequenced using the Proton-Ion sequencing instrument.
Results: In total, 4.13 Gb were mapped with 94% on target and base reads with 370 reads per amplicon and overall average base coverage depth of 325. In the 243 samples, a total of 51 variants (including 2 homozygous CACNA1C and SCN5A homozygous mutations) with an overall clinical sensitivity of 51/242 (21%) was found. Sub-panel diagnosis based clinical sensitivity was 32% for cardiomyopathy, 10% for congenital heart disease, 31% for arrhythmias and 29% for aortic aneurysms/Marfan syndrome.
Conclusion: This custom made, comprehensive cardiac gene panel appears to have a clinical sensitivity comparable to that of the current WES with the advantage of faster turnaround time and far fewer variants to analyse. Only in 6% of the genes on this panel were relevant DNA sequence variants identified. Hence, a tiered approach starting with a custom gene panel followed by WES is probably a more efficient clinical approach.

PM05.56
Homozygous, and compound heterozygous mutation in 3 Turkish family with Jervell and Lange-Nielsen Syndrome

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Long QT syndrome is one of the most common congenital cardiac ion channel disorder that the morbidity and mortality rate can be decreased by an early diagnosis and proper treatment. Cardiac repolarization abnormality that is characterized by prolonged OT interval and propensity for ventricular tachycardia of the torsades de pointes type are characteristics of the disease. This syndrome represents high risk for presyncope, syncope, cardiac arrest and sudden death. Jervell and Lange-Nielsen syndrome (JLNS) is recessive form of long QT syndromes with additional finding of profound sensorineural hearing loss. JLNS has been shown to occur due to homozygous and compound heterozygous mutations in KCNQ1 or KCNE1. Pathogenic mutations in the KCNQ1 gene were detected in all our JLNS cases. Index cases of 3 families were 2 month yr, 3.5 yr old female and 3-yr old male who visited the hospital due to intrauterine bradicardia, recurrent seizures/syncope, cardiac murmur, respectively and had all congenital sensorineural deafness. Their electrocardiograms revealed a markedly prolonged QT interval. The sequence analysis of the probands revealed the presence of compound heterozygous mutation ([(c.477+1G>A) + (c.520C>T, p.R174C)] and homozygous missence mutations (c.728 G>A, p. R243H), (1097G>A, p.R366Q), respectively. Heterozygous mutation in KCNQ1 was identified on the maternal, paternal and sibling sides. Homozygous mutation was identified in 3-yr old male's sister and cousin also. Interestingly even if her QT is long she had intact hearing. β-blocker therapy was initiated to all affected ones. Asymptomatic heterozygous family members were taken to a clinical follow up. Clinical and moleular findings will be discussed to further enlighten the genotype-phenotype association.

PS05.57

A common variant associated with coronary artery disease increases atheroma size within atherosclerotic plaques of patients with carotid stenosis

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Background
To date, genome-wide association studies have identified 2 susceptibility loci for large artery ischemic stroke and 45 loci for coronary artery disease (CAD). We investigated whether these SNPs affected atherosclerotic plaque characteristics in the Athero-Express Biobank Study (AE, www.atheroexpress.nl) of patients with clinically significant carotid stenosis, from whom plaque specimens have been histologically analyzed.
Methods
We genotyped 1,439 patients from the AE and imputed missing genotypes using HapMap. We tested the 47 SNPs for association to macrophages numbers, smooth muscle cells (SMCs) numbers, neovascularization, intraplaque hemorrhage (IPH), atheroma size, calcification, and collagen content, using linear or logistic regression, correcting for age, sex, year of surgery, array type, and 10 principal components. We considered a significant association at p < 1.5 x 10-4 after Bonferroni correction for the number of variants and phenotypes tested.
Results
The most significant association was found for rs12539895 (coded allele frequency = 0.75, odds ratio = 1.59 per C allele, p = 9.0 x 10-6) with percentage of atheroma. This direction of effect was such that the same allele increases atheroma size and CAD risk. Other variants were nominally significantly associated with macrophages (rs17609940, p = 0.0457), SMCs (rs11203042, p = 0.0478), IPH (rs11203042, p = 0.0020), and atheroma (rs2023938, p = 0.0331; rs445925, p =0.0082), all with effect directions consistent with their reported effect on CAD.
Conclusion
Of 47 previously associated variants, one variant (rs12539895) was significantly associated with atheroma size in patients with carotid atherosclerosis. Further research is warranted to better understand underlying mechanisms.

PM05.58

Role of ceramide synthase 5 gene (*LASS/CERS5*) in molecular pathogenesis of atherosclerosis.

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Ceramide, the backbone of sphingolipids, is the key component which affects atherosclerotic changes through its important second-messenger role. In previous studies, the protective role of *AMPK* gene with its regulated genes in atherosclerosis and hypertension is shown. *LASS5 (CerS5*) gene takes place in ceramid synthesis and has an indirect effect on *AMPK* gene. In this study, our aim was to identify *LASS5* gene function in athrerosclerosis. Briefly, *LASS5* gene specific siRNA mediated gene silencing was performed in HUVEC and subsequently the differencial expession *AMPK* regulated genes were analyzed. Then, HUVEC cells were treated with *AMPK* inhibitor or activator in order to examine the relation of change in gene expression levels with *AMPK* activity changes. As a result, we have identified a noval physiological function of *LASS5*. The treatment of *AMPK* inhibitor (Compound C) ligand significantly increased *LASS5* mRNA expression in HUVEC. Downregulation of *LASS5* was found to attenuate ceramide production, and increased the expression of *eNOS* and *KLF2*. In addition, we observed that presence of *LASS5* siRNA induced the expression of *KLF2* genes, and that this induction was partially prevented by Compound C. On the contrary, we observed that co-presence of *LASS5* siRNA and *AMPK* activator (AICAR) increased the expressions of *eNOS* and *KLF2*.
In summary, this is the first study demonstrated that *LASS5* was involved in the negative regulation of atherosclerosis related genes, namely *eNOS* and *KLF2*. These findings provide an insight into the molecular mechanism of atherosclerosis and also have importance for the development of potential therapeutic agents in the treatment of atherosclerosis.

PS05.59

Use of gene panels for the diagnosis of cardiac arrhythmia

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Cardiac arrhythmia occurs when the electrical impulses of the heart become disrupted. This can lead to a variety of syndromes including Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT), Brugada Syndrome(BS), and Long QT Syndrome(LQTS). These disorders are all genetically and clinically heterogeneous with significant phenotypic and genetic overlap. Incomplete penetrance and variable expressivity adds further complication. In cases of sudden cardiac death where no record of the heart's electrical activity exists, it is extremely difficult to predict the correct molecular diagnosis.
Next Generation Sequencing (NGS) technology allows the provision of clinically relevant and cost effective solutions for molecular diagnosis. We have developed and validated an NGS panel of 43 genes which includes four subpanels for arrhythmic disorders CPVT, BS, LQTS and arrhythmia molecular autopsy. The strategy used is Haloplex enrichment technology (Agilent Technologies) followed by paired end sequencing on the MiSeq (Illumina) and an in-house analysis pipeline, "HAPPy". The pipeline is fully flexible, thus allowing any combination of genes on the panel to be analysed together.
The results of the first six months of this new service will be presented to demonstrate the analytical and clinical sensitivity of our testing strategy. Emphasis will be placed on the interpretation and clinical follow up of unclassified variants in the diagnostic setting e.g. a non-synonymous potentially pathogenic variant in *CALM1* in a patient with young onset severe Brugada syndrome. *CALM1* pathogenic variants have previously only been reported in LQTS and CPVT.

PM05.60

Loss of Y in blood and plaque is associated with atherosclerotic disease in men undergoing carotid endarterectomy

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Introduction:
The Y chromosome has long been considered genomic wasteland with few genes only implicated in sex determination. However, recent studies found an immunoregulatory role for Y and a relation between loss of Y (LOY) and a higher cancer risk and mortality. Given the involvement of immune cells in atherosclerosis, we hypothesized that LOY is associated with cardiovascular disease (CVD) phenotypes in men undergoing carotid endarterectomy (CEA).
Materials and Methods:
LOY was quantified in blood and plaque from raw intensity genotyping data in a cohort of 582 men in the Athero-Express Study. We tested LOY for association with CVD phenotypes, atherosclerotic plaque phenotypes and outcomes during 3-year follow-up.
Results:
LOY in 228 blood and 135 plaque samples was negatively associated with age in both blood (beta=-0.03/10yr, p=3.34*10-5) and plaque (beta=-0

.02/10yr, p=0.001). Quartiles of LOY were associated with contralateral arterial stenosis in blood (beta=-0.83,p=0.02) and peripheral arterial occlusive disease (beta=0.17,p=0.03) and stroke history (beta=-0.20,p=0.3) in plaque. LOY was not associated with plaque phenotypes. Event-free survival for major cardiovascular disease events was lowest in the quartile with the most Y-loss in blood (log-rank test p-value=0.03). Although LOY was correlated between blood and plaque the variance was higher in blood suggestive for more Y instability in blood as compared to plaque.
Conclusion:
LOY in blood and plaque is associated with hallmarks of atherosclerotic disease, but not with plaque phenotype in men undergoing CEA. Furthermore, men with LOY in blood had more major cardiovascular events during follow-up.

PS05.61
Losartan therapy in Marfan syndrome is only effective in patients with haploinsufficiency of FBN1

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Introduction: Patients with Marfan syndrome - caused by FBN1 mutations - have an increased risk of life-threatening aortic complications. It has been shown that losartan reduces aortic dilation rate in these patients. The response to losartan treatment, however, was highly variable between individuals. Here we investigate whether there is a difference in Losartan effectiveness in genetically classified subgroups.
Methods: In this predefined sub-study of the COMPARE trial, we classified FBN1 mutations into: 1) Dominant negative mutations leading to a mutated fibrillin-1 protein incorporated in the extracellular matrix, 2) Haploinsufficient mutations leading to decreased amount of normal fibrillin-1 protein. The response to losartan therapy based on aortic root dilatation rate was compared between the two groups.
Results: Baseline characteristics between treatment groups were similar. Overall, losartan significantly reduced aortic root dilatation rate. However, losartan only reduced aortic root dilatation rate in haploinsufficient patients and not in dominant negative patients.
Conclusion: Marfan patients with haploinsufficient FBN1 mutations are more responsive to losartan therapy with respect to inhibition of aortic root dilatation rate compared to dominant negative patients. In order to predict response on losartan therapy, mutation classification should be performed for all Marfan patients. More research for novel treatment strategies is needed in Marfan patients with a dominant negative FBN1 mutation

Aortic root dilatation in Marfan patients

Type of FBN1 mutation	Aortic root dilatation	in mm/3 years	
	No losartan	+ losartan	p-value
total	1.3 ± 1.5 (n=59)	0.8 ± 1.4 (n=58)	0.009
dominant negative	1.2 ± 1.7 (n=38)	0.8 ± 1.3 (n=41)	0.197
haploinsufficient	1.8 ± 1.5 (n=21)	0.5 ± 0.8 (n=17)	0.001

PM05.62
Dissecting metabolic syndrome-related QTLs on rat chromosome 4 via eleven new double congenic rat strains

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Rat chromosome 4 comprises several regions frequently associated with metabolic syndrome features. In order to dissect the individual QTLs we derived 11 new double congenic strains by introgressing distinct segments of rat chromosome 4 (RNO4) of spontaneously hypertensive rat (SHR) origin into BN-Lx (Brown Norway) genomic background.
Methods: We defined the spans of respective differential segment using 79 microsatellite markers. 16-week-old male rats (n=6/strain) were fed a standard diet. We assessed comprehensively the morphometric and metabolic profiles of all groups, including glucose tolerance tests, levels of insulin, adiponectin and concentrations of triglycerides and cholesterol in 20 lipoprotein fractions. One-way ANOVA with STRAIN and as major factor was used.
Results: Fasting glucose (ANOVA p = 0.00014) was considerably lower in BN-Lx.SHR4(A2m) (2.3 ± 0.24 mmol/l) and BN-Lx.SHR4(Il6 Lmbr Rat7 (Rat248-Rat150)) (3.66 ± 0.20 mmol/l) compared to BN-Lx (4.8 ± 0.42 mmol/l). Glucose tolerance was improved in BN-Lx.SHR4(A2m) (area under the glycemic curve fell by 42,1% compared to BN-Lx) but deteriorated in BN-Lx.SHR4(Pparg) (area under the glycemic curve rose by 17% compared to BN-Lx). We observed most striking shifts of TG and cholesterol concentrations profile in strains BN-Lx.SHR4(A2m), BN-Lx.SHR4(CD 36) and BN-Lx.SHR4(Pparg). In silico sequence comparison of differential segments in these strains revealed several genes harboring mutations in SHR.
Conclusion: Using derivation of series of double congenic strains, we have validated several metabolic syndrome-related QTLs and identified potential sequence variants underlying these loci.
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PS05.63
Clinical diversity of MYH7- novel mutations and novel insights into pathogenesis.

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Introduction
Mutations in the myosin heavy chain 7, cardiac muscle, beta gene (*MYH7*) are an important cause of both primary cardiomyopathy and skeletal myopathy. Previous data have established interesting correlations between various phenotypical features described in *MYH7* related disease (*MYH*-RD) and the specific site of the mutations within the gene.
Case Series
Three pedigrees with cardiomyopathy were studied. Pedigree 1, a Jewish Yemenite family, had 11 members with echocardiographic evidence of non-compaction cardiomyopathy and varying degrees of Epstein anomaly. This phenotype comprises approximately 4% of *MYH7*-RD. Pedigree 2 had a single member suffering from congenital dilated cardiomyopathy (DCM) and skeletal myopathy consistent with congenital fiber-type disproportion. The presentation of DCM and concomitant skeletal involvement congenitally is relatively uncommon. The third pedigree included 2 deceased siblings with DCM and their deceased first cousin with reported restrictive cardiomyopathy.
Results and Discussion
Direct sequencing of *MYH7* (NM_000257.3) in these pedigrees identified two novel mutations; c.1859T>C (p.Leu620Pro) in pedigree 1 and c.5655+1G>A in pedigree 2, predicted to disrupt the donor splice site of exon 38. These mutations expand the known genotypic spectrum of these relatively rare phenotypes of *MYH*-RD. Mutation analysis of the one available patient from pedigree 3 identified a compound heterozygous mutation c. 427C>T (p.Arg143Trp) and c. 4588C>T (p.Arg1530X). Interestingly, c. 427C>T was previously described as causing hypertrophic cardiomyopathy via a dominant negative effect, yet the proband's healthy mother and aunt carried this mutation. This may alter our view on disease pathogenesis, suggesting the contribution of nonsense mutations in the resulting disease phenotype.

PM05.64
Two novel MYLK nonsense mutations causing thoracic aortic aneurysms/dissections in patients without apparent family history

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Introduction: Thoracic aortic aneurysm and dissection (TAAD) is a genetically heterogeneous disorder representing a frequent cause of morbidity and mortality in the western world. To date, only two heterozygous loss-of-function mutations have been described in *MYLK* (myosin light chain kinase) causing familial aortic disease with little to no aortic enlargement prior to dissection. Here, we have aimed to expand the phenotypical spectrum associated with *MYLK* mutations.
Methods and results: After the application of a next-generation sequencing based TAAD gene panel in a cohort of 359 syndromic and non-syndromic TAAD patients, we identified two novel heterozygous *MYLK* mutations leading to a premature stop codon. Two female patients, with nonsense mutations at amino acids p.Arg1458 and p.Arg1487, presented with type B aortic dissection at ages 47 and 49 years, respectively. Both patients had a longstanding history of hypertension. Physical exam revealed unilateral iris flocculi in one, whereas the other woman presented with several systemic connective tissue findings. Remarkably, none of the patients had a family

history of aortic aneurysms or dissections.
Conclusions: Two novel heterozygous loss-of-function *MYLK* mutations have been identified. In addition to vascular findings, patients showed variable systemic features. The current report doubles the number of known *MYLK* mutations and significantly informs the further clinical delineation of the *MYLK* phenotypic spectrum.

PS05.65
Coincidence of ANKRD1, TMEM43 and PKP2 mutations in patient with ARVD

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Background. Cardiomyopathy is characterized by mechanical or electrical dysfunction of cardiac muscle and it is a known risk factor of sudden cardiac death. More than hundreds of variant in 84 genes have been associated with inherited cardiomyopathy. A high number and variability of involved genes complicate diagnosis of cardiomyopathy. New technology of targeted next generation sequencing has a great potential in diagnostic settings of cardiomyopathies. It allows us to analyze several genes in parallel, which entails that several variants of potentially pathological or even unknown clinical significance are detected.
Methods. We applied next generation sequencing of 46 genes which have previously been shown to be associated with different types of cardiomyopathies (TruSight Cardiomyopathy Panel, Illumina) on group of unrelated patients with DCM, ARVC and ventricular fibrillation.
Results. The summary of the results of our analysis of the entire group of patients will be presented. A case report is selected, in which we present a patient with serious symptoms of Arrhythmogenic right ventricular dysplasia (ARVD), including ventricular tachycardia and sudden cardiac arrest, in which we revealed three potentially pathological mutations in three different genes. Two missense mutations in *ANKRD1*and *TMEM43* gene and one mutation in *PKP2*, which causes aberrant splicing were detected.
Conclusions. We hypothesized that the combination of these three pathological mutations could be the reason for the lethal course of the disease. Moreover, we assume that mutations in *ANKRD1* gene, which were previously shown to be responsible for dilated cardiomyopathy and hypertrophic cardiomyopathy, could be associated also with ARVD.
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PM05.66
Rare variants residing in the ADAMTS13 von Willebrand factor (VWF) binding domain contribute to pediatric stroke susceptibility

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Recently we reported a gene network of ADAMTS (A Disintegrin-like and Metalloprotease with Thrombospondin motifs) genes as central component of the genetic risk contributing to pediatric stroke. ADAMTS13 is a prime example for such a key component as it cleaves VWF multimers, reduces platelet adhesion and aggregation and down-regulates thrombus formation and inflammation. Here we characterized the genetic architecture of ADAMTS13 through targeted next generation sequencing of 48 affected children and their unaffected siblings. In total, we identified 226 variants in ADAMTS13, 36 of which are rare and resist filtering for unambiguous allele calling. For genotyping in 270 trios, 21 common variants covering the complete ADAMTS13 gene were selected based on significance in the sib-ship disequilibrium test ($p<0.05$) or protein altering properties. Transmission disequilibrium testing was performed for affection status and ADAMTS13 activity levels using PLINK and FBAT, respectively. 10 SNPs were significantly associated with pediatric stroke ($p<0.05$ to <0.001), two of which (rs2285489, rs28793911) were also significantly associated with ADAMTS13 levels ($p=0.0004$ and $p=0.0092$). The C-alpha collapsing test implemented in AssotesteR was used to assess significance across the set of 36 rare variants yielding a significant association ($p=5.73x10-6$). Haplotype association using a sliding window approach assigns this association to the ADAMTS13 VWF-binding domain ($p=1.2$ x10-4). Our data provide a link between the genetic architecture of ADAMTS13, ADAMTS13 levels and stroke risk. Altogether, these studies render ADAMTS13 an attractive candidate for functional studies and a potential diagnostic kit that incorporates genetic variation as predictors for stroke risk.

PS05.67
Molecular screening of NKX2.5 in two Moroccan cohorts with atrial septal defect (ASD) and tetralogy of Fallot (TOF)

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Congenital heart disease (CHD) is one of the most frequent disorders observed at birth, affecting more than 1/100 live births. Among all CHDs, Atrial Septal Defect (ASD) and Tetralogy of Fallot (TOF) account for approximately 10% and 7% respectively. The previous studies have shown that mutations in NKX2.5 were associated with these two disorders. The aim of this study was to screen the NKX2.5 mutations in two series of Moroccan patients affected respectively by ASD and TOF.
Thirty four patients were recruited in HASSAN II university hospital of Fez; DNAs were extracted from Blood samples. Then, all samples have undergone amplification by PCR and direct sequencing of NKX2.5 coding regions. The obtained sequences were analysed by Bioinformatic alignment tools.
We have detected 3 mutations in ASD cohort and 2 mutations in TOF cohort which represents respectively 10.5% and 15.3% of the studied cohorts. These prevalences are much higher than those reported in the previous studies, which requires more studies in larger Moroccan cohorts. Moreover, we have found that the polymorphism c.63A>G is quite common in the Moroccan population with prevalence around 75%.
Grant References: Faculty of Medicine and Pharmacy of Fez – Hassan II University Hospital of Fez, Morocco.

PM05.68
Novel desmoplakin splice site and TMEM43 missense variant presenting with noncompaction cardiomyopathy (NCCM)

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Noncompaction cardiomyopathy is a genetic cardiomyopathy associated with defects in sarcomere genes, in particular MYH7 and Titin. NCCM is characterized by distinct morphologic changes with hypertrabeculation and deep intertrabecular recesses of the left ventricular wall. In arrhytmogenic right ventricular cardiomyopathy loss of myocytes and fibro-fatty tissue replacement are caused by mutations in genes affecting the function of the desmosomal complex resulting in comprised cell-to-cell adhesion and differential signaling. Among the ARVC genes the DSP gene has shown to have a strong effect on left ventricular function, in contrary to TMEM43. Mutations in ARVC genes are rarely observed in NCCM. So far only two heterozygous truncating DSP variants (c.5208_5209delAG and c.1339C>T) and no TMEM43 variants have been associated with NCCM.
We found a DSP splice site mutation (c.3084+1G>A) classified as pathogenic, in a women who had 2 children, diagnosed with NCCM at age 33 years. She suffered from palpitations and repeated syncope, the ECG showed sustained ventricle tachycardia with right bundle branch morphology. After cardioversion the resting ECG didn't show inverted T-waves or epsilonwaves as expected in ARVC.
Cardiac echo showed a dilated right ventricle without dyskinesia and a hypertrabeculated left ventricle fulfilling the Jenni criteria for NCCM. The cardiac MRI showed a dilated right ventricle with hypertrabeculation, without dyskinesia. Left ventricle showed a decreased ejection fraction of 40% with noncompacted versus compacted ratio of 4,6. The patient met diagnostic criteria for NCCM, not for ARVC. These observations suggest genetic overlap between ARVC and NCCM and further studies are needed to elucidate the underlying shared pathophysiologic mechanism.
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PS05.69
Genetics in noncompaction cardiomyopathy(NCCM)

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Introduction: Noncompaction cardiomyopathy (NCCM) is a genetic cardiomyopathy, characterized by excessive trabeculations with deep recesses of the left ventricular wall. We present the results of genetic analysis in a large cohort of NCCM patients.
Method: The study included all NCCM index patients diagnosed and tested from 2005 till 2015 in our medical center. Current molecular testing for genetic defects is performed using novel next generation sequencing techniques of a panel of 48 cardiomyopathy related genes. Genetic sequence

variants in the cardiomyopathy genes were classified for pathogenic effect according to the current five category diagnostic criteria.
Results: The study include 128 index NCCM patients. Pathogenic or likely pathogenic variants (class 4 and 5) were identified in 40% (49/128) of the patients, including two or more (likely) variants mutations in 8 % (4/49). MYH7 mutations were the most frequent, affecting 16% (21/128) of the patients. The titin gene appeared to be an important cause of NCCM since we found truncating variants classified as likely pathogenic in 10% (7/67) of the tested patients. In two patients (2/67) the MIB1 gene, known to regulated embryologic compaction of the ventricular wall, was involved. In 30% (38/128) of the patients a variant of unknown clinical significance (class3) was found.
Conclusion: Extensive genetic testing detected a (likely) pathogenic variant in 40% of NCCM patients. The improving molecular diagnostics for cardiomyopathies have a profound impact on counseling and screening of families of NCCM patients. Further studies are needed to understand the contribution of genetic factors to this disease.
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PM05.70

Paediatric cardiomyopathy (PC); the utility of a 71 gene NGS diagnostic panel to detect variants in rare cardiac genes.

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Paediatric cardiomyopathy (PC) can present in infancy with cardiac failure and sudden death. Considerable clinical and genetic heterogeneity exists within and between cardiomyopathy families hence cost effective gene panel testing aids diagnosis and elucidates complex clinical presentations. A 71 PC gene panel (Agilent SureSelect) has been validated (UKGTN approved) with coverage of 99.9% at 30X, and data analysis using an in-house bioinformatics pipeline based on the Broad Institute and Geneticist Assistant (SoftGenetics).
To date 37 patients have been tested, including paediatric cases; patients negative for other genes and patients who have phenotypic incompatibility with their reported pathogenic variant where digenic inheritance is suspected. 32/37 (86%) patients have at least one potentially pathogenic variant. In one family with HCM, a *MYBPC3* variant c.1505G>A, p.(Arg502Gln) and a previously reported *NEBL* pathogenic variant c.180G>C, p.(Lys60Asn) (nebulette protein) were detected in a severely affected male demonstrating that multiple variants can explain phenotypic severity. The utility of testing rare genes is exemplified by: 1) A teenager with LV dilation and FH of sudden death who was heterozygous for a *RMB20 (*RNA binding protein) variant c.1907G>A, p.(Arg636His), previously reported with severe familial DCM; 2) a patient with congenital heart block, LV dilation and FH was heterozygous for a novel likely pathogenic *MYH6* (alpha heavy chain subunit) variant c.3578C>T, p.(Ala1193Val). Furthermore, a novel heterozygous *TTN* A-band frameshift variant was identified in an infantile DCM patient; recent data suggests *TTN* frameshift variants have not been reported in infantile cardiomyopathy.
An audit of this patient cohort will be presented, illustrated by interesting cases.

PS05.71

Genome-wide analysis of parent-of-origin effects on cardio-metabolic traits

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Parent-of-origin effect (POE) is an epigenetic phenomenon that makes two alleles at a locus functionally non-equivalent and manifested as phenotypic differences between reciprocal heterozygotes. We performed parental origin-specific genome-wide association analyses for cardio-metabolic traits to identify genetic variants exhibiting POE.
The study included 617 individuals with at least one parent genotyped from 150 large Finnish dyslipidemia families, and were genotyped at ~550,000 genetic variants using Illumina CoreExome beadchip array. Parental origins of alleles were determined using long-range phasing and genealogy as implemented in Alphaphase 1.1. POE was assessed using mixed linear models (adjusted for age, sex and relatedness) evaluating (1) phenotypic differences between reciprocal heterozygotes, and (2) effect of maternally and paternally inherited alleles separately.
Analysis revealed potential POEs on cardio-metabolic traits in seven loci (*NTNG1, CELF4, CLDN14, CDKN1A, PMP22-TEKT3, GPR56, EIF4E)* at *P* value $<1.0\times10^{-7}$. The strongest signal for parental origin specific association was obtained at rs531284 near *NTNG1* for fasting glucose levels. The maternally inherited minor allele at rs531284 showed association with reduced fasting glucose level [$_{mat}$=-0.67 s.d. units, $P_{mat}=7.8\times10^{-8}$] whereas paternal allele was not associated. At rs2249508 near *CLDN14*, paternal specific association was observed with LDL particle size [$_{pat}$=-0.55 s.d. units, $P_{pat}=9.8\times10^{-8}$]. These preliminary associations are now being tested for replication on further family studies.
In conclusion, the study indicates POE of genetic variants on glucose and lipid-related traits and suggests that this approach could help to identify new susceptibility genes for complex disorders.
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PM05.72

Association of R279Q and C1562T polymorphisms of Matrix metallopeptidase-9 (MM-9) gene and increased risk for myocardial infarction in patients with premature coronary artery disease among Iranian population

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Background: The present study assessed the relationship between these two polymorphisms of the matrix metalloproteinase-9 (MMP9) gene (R279Q A/G polymorphism (rs17576) and C1562T polymorphism (rs3918242)) and occurrence of myocardial infarction in patients with premature coronary artery disease.
Methods: Our prospective study included 1000 patients with the final diagnosis of premature CAD and classified into two groups with history of MI (n = 461) and without of MI (n = 539). The polymorphism variants were determined by PCR-RFLP and High Resolution Melting techniques. Among study samples, 640 were followed with a median follow-up time 59 months for determining association of major adverse cardiac events (MACE) and polymorphisms.
Results: The prevalence of wild, heterozygous and mutant genotypes of R279Q polymorphism in MI group was 14.5%, 57.3%, and 28.2% and in non-MI group was 36.9%, 38.4%, and 24.7%, respectively with a considerable difference ($p < 0.001$). There was a significant difference in the prevalence of wild, heterozygous and mutant genotypes of C1562T polymorphisms in MI group (12.4%, 41.2%, and 46.4%, respectively) and in non-MI group (46.8%, 38.6%, and 14.7%, respectively) ($p < 0.001$). After adjustment for covariates, significant differences were revealed in genotypes of R279Q and C1562T polymorphisms between MI and non-MI groups. No difference was found in total-MACE free survival rate in MI patients and also in non-MI patients with different genotypes of R279Q and C1562T polymorphisms.
Conclusion: C1562T and R279Q polymorphisms of MMP-9 gene can be associated with the susceptibility to MI in premature CAD patients. However, these polymorphisms may not be able to predict long-term cardiac events in these patients.

PS05.73

Pathogenic potential of FOXC2 mutations identified in patients with primary lymphedema

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Dominant mutations in the FOXC2 gene cause a form of lymphedema that usually develops around puberty. In a large percentage of patients, lymphedema is accompanied by distichiasis and varicose veins. Other clinical abnormalities, such as cardiac defects, epidural cysts, ptosis and cleft palate have also been reported but are less common. FOXC2 is a member of the forkhead/winged-helix family of transcription factors and plays essential role in different developmental pathways and physiological processes. Most of FOXC2 mutations described so far either truncate the protein or are missense mutations in the forkhead domain causing a loss of function. The

haplo-insufficiency is associated with generalized hyperplasia of lymphatic system in mice as well as in humans.
We previously described six unrelated families with primary lymphedema in which patients showed different FOXC2 mutations. Of those, 4 were missense mutations, one a frame-shift mutation and the last one a stop mutation; all of them were located outside of the forkhead domain. To evaluate their pathogenic potential, we now have examined their subcellular localization and performed a transactivation assay using a luciferase construct with FOXC1 response elements. All FOXC2 mutated proteins are able to localize correctly into the nucleus. Alf of them present reduced while the remaining increased transcriptional ability, ranging from a complete loss to a significant gain of function. Our data suggest that the unbalanced FOXC2 activity causes a dramatic perturbation of lymphatic vessel formation leading to lymphedema.

PM05.74
Molecular and functional characterization of BMPR2 gene in Pulmonary Arterial Hypertension

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Pulmonary Arterial Hypertension (PAH; OMIM 178600, ORPHA 422) is an autosomal dominant disease characterized by pulmonary vascular resistance increase, vascular remodelling and right heart failure. Symptoms of PAH include fatigue, shortness of breath and syncope. Several genes have been related to HAP, the most implicated gene is BMPR2 with more than 300 mutations described, 80% in Familial HAP and 20-40% in Idiopatic HAP. However, some of the functional implications for these mutations are unknown. The aim was to characterize the pathological mechanism of several mutations in BMPR2 and correlate them with the clinical spectrum of the disease.
Variants were selected from an in silico analysis. mRNA expression studies were performed using pSPL3 vector to confirm splicing alterations, studies of subcellular localization were performed using pEGFP-N1 vector and luciferase assay were performed using pGL3-Bassic vector. Correlation genotype-phenotype was performed using Spss v.19 software.
Nine out of 24 analyzed changes affect mRNA processing (p.S52Sfs*2, p.C84F, p.P138A, p.R211R, p.Y218*, p.V278V, p.W298*, p.P327P, p.K467R) and 4 out of 18 variants affects the subcellular localization (p.S52Sfs*2, p.C84F, p.Y218*, W298*). Five changes were identified at 5´UTR region and four of them produced a gene expression decrease of >50%. Patients with these pathogenic mutations showed an early age of diagnosis (p=0.047) and greater sPaP (p=0.042).
Molecular and functional studies have allowed us to check the real implication of the mutation and to correlate the role in the phisiopathological pathway of the disease. Likewise, these mutations predispose individuals to a more severe phenotype and this group shown a early age at diagnosis.

PS05.75
Extending the phenotypic spectrum of *RYR2* mutations: mind the brain!

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Introduction: Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a rare inherited cardiac disorder characterized by adrenergically-mediated ventricular arrhythmias. The most common autosomal dominant form of CPVT is caused by mutations in the cardiac ryanodine receptor (*RYR2*) gene. Apart from the myocardium, this calcium release channel is highly expressed in the brain. Here we studied the prevalence and characteristics of neuropsychiatric disorders in patients with CPVT.

Methods: We included all CPVT patients with a pathogenic *RYR2* mutation and intellectual disability (ID) from 4 tertiary referral centers in the Netherlands and Japan. ID was defined as significant impairment of cognitive and adaptive functions with onset before the age of 18.

Results: Among 250 CPVT patients carrying a *RYR2* mutation, we identified 18 patients with ID (7.2% compared to 1% of the general population). The *RYR2* mutations (15 missense and 1 splice site) clustered in the known hot-spot regions. Two mutations have been previously described in patients without reported ID. In 9 out of 14 patients studied (64%) the mutation appeared *de novo*. The severity of ID ranged from mild to severe, and was often accompanied by behavioral disorders including autism and attention deficit hyperactivity disorder. Of note, the majority of patients displayed marked supraventricular and ventricular arrhythmias, with a high incidence (39%) of serious arrhythmic events during follow-up including 3 fatal events.

Conclusions: ID is more prevalent among *RYR2* positive CPVT patients than in the general population, and is associated with a malignant cardiac phenotype.

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PM05.76
MSVA - mutation and sequence variation analysis software

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In order to assist the integration of patient data on rare diseases with tools for statistical analysis, we created advanced form of a patient/variation/symptom database for the use on desktop computers and particularly for installation on a web server. The secured web form of the database is controlled via assignment of individual logins and passwords and security of the data is achieved by fine layering of data access/editing permissions and, at the same time, is adjusted to enable sharing of the data which is a crucial function of the database.
Structure of the underlining MySQL database is designed in the way that each gene is represented by a separate table with patients/variations (rows) and their symptoms (columns), and the content of the tables is called by built-in statistical functions to perform inference. Database administrator is able to add new genes (tables) while the users of the database create new records (rows) of patients/variations and fill in their symptoms. We will present current structure of the user interface of the software and details of the included statistical tools which enable to test hypotheses on subsets of patients and subsets of their variations and symptoms. The current version contains tables for genes with structural functions in the cytoskeleton and nucleoskeleton and patient data including symptoms of diseases like dystrophy, cardiomyopathy, lipodystrophy etc. This work is supported by the Czech Ministry of Industry and Trade (MPO FR TI 3 588) and the Czech Ministry of Education (COST MSMT LD12063).

PS05.77
MCEMP1 expression as a biomarkers of stroke diagnosis and prognosis

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Introduction: Stroke is a leading cause of death and functional disability. A limitation in the current evaluation and treatment of patients with suspected stroke is the lack of a rapid diagnostic test. A blood-based biomarker of acute stroke may expedite stroke diagnosis and prognosis. As such we sought to identify genes expressed in peripheral blood that are associated with (1) stroke, (2) primary stroke type, ischemic or hemorrhagic, and (3) stroke prognosis.
Methods: Stroke cases and controls were recruited from a subset of centers involved in the international INTERSTROKE study. Patients were consecutively assigned to the biomarker discovery cohort (N=302) or validation cohort (N=62). In the discovery cohort transcriptome-wide peripheral blood gene expression was assessed using the Illumina HumanRef-8 v4 bead chip. Significant genes were validated using qPCR in the validation cohort.

Results: Expression of a single gene, MCEMP1, was sufficient to distinguish between stroke cases and controls (FC=2.4, P-value = 8.2 x 10-22, AUC=0.812). MCEMP1 expression also differentiated between ischemic and hemorrhagic stroke cases (AUC=0.783). Furthermore, MCEMP1 expression was significantly associated with one-month outcome, measured as Modified Rankin Score (MRS), after adjustment for available risk factors, primary stroke type and baseline MRS (FC=1.29, P-value = 1.09 x 10-5). Finally the associations between MCEMP1 and stroke and one-month MRS were independently confirmed in the validation cohort.
Conclusion: Peripheral blood expression of MCEMP1 may have utility as a diagnostic test for acute stroke, in distinguishing ischemic stroke from intracerebral hemorrhage and a prognostic biomarker of outcome.

PM05.78
NOTCH3 mutations and risk of young stroke in INTERSTROKE

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Introduction: CADASIL is a rare Mendelian stroke syndrome due to mutations in the NOTCH3 gene. CADASIL syndrome is characterized by ischemic stroke, migraine, and cognitive impairment in young patients with a family history of stroke. Whether mutations in NOTCH3 or other Mendelian stroke genes are risk factors in young patients unselected for syndromic features is unknown.
Methods: Using exome sequencing, 10 Mendelian small-vessel stroke genes were analyzed in young unrelated stroke cases (mean age: 47.6 years), comprising 100 intracerebral hemorrhage (ICH) and 85 small-vessel ischemic stroke (SVIS), and 185 matched controls from INTERSTROKE, a large international case-control study of stroke.
Results: Canonical CADASIL mutations were exclusive to cases and associated with risk of all stroke (P=0.02), explaining 3.2%, 3%, and 3.5% of all stroke, ICH, and SVIS, respectively. We estimated that CADASIL mutation carriers are at high risk of ICH (OR=9.36; P=0.02) and that the population prevalence of CADASIL is 340 per 100,000 individuals. Non-CADASIL NOTCH3 mutations were also associated with risk of ICH (OR=2.66; P=0.03) and were present in 19% of ICH cases and 8% of controls. Secondary features of CADASIL (migraine, depression, psychiatric disturbances, subclinical infarcts, parental history of stroke) were not more frequent in NOTCH3 mutation carriers as compared to non-carriers. No significant associations were observed for the other 9 genes.
Conclusion: NOTCH3 mutations are important risk factors for ICH in young people. CADASIL mutations pose high risk to ICH and their prevalence may be underestimated. Genetic screening of NOTCH3 should be considered in young stroke patients.

PS05.79
Sudden cardiac arrest and rare genetic variants in the community

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Sudden cardiac arrest (SCA) ranks among the most common causes of death worldwide. SCA incidence in the community varies between 0.6 and >1.4 per 1,000 individuals. Because SCA mostly occurs in individuals without previously known cardiac disease, the identification of patients at risk for SCA could save many lives. In unselected SCA victims from the community, common genetic variants (which are not disease-causing per se, but may increase susceptibility to VF) have recently also been associated with increased SCA risk. However, whether rare genetic variants contribute to SCA risk in the community is largely unexplored.
We here investigated the involvement of rare genetic variants in SCA risk at the population level, by studying the prevalence of six founder genetic variants present in the Dutch population (PLN-p.Arg14del, MYBPC3-p.Trp792fsX17, MYBPC3-p.Arg943X and MYBPC3-p.Pro955fsX95, PKP2-p.Arg79X and the Chr7q36 IVF risk-haplotype) in a cohort of 1440 unselected Dutch SCA victims included in the AmsteRdam REsuscitation Study (AREST). The six studied founder mutations were found to be more prevalent, 1.1% carried one of the mutations, in our cohort of SCA patients than in a locally matched reference cohort (0.4%, n=1379, p<0.05) and in publicly available reference variant databases (e.g. the Genome of the Netherlands cohort, GoNL, 0%, n= 500 p<0.02). This finding provides proof-of-concept for the notion that rare genetic variants contribute to SCA risk in the community.
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PM05.80
Family follow up and genetic testing in sudden cardiac death with a structurally normal heart

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Aims:
To assess the efficiency of the Familial Arrhythmic Network Scotland (FANS) in following up the families, and to assess the effectiveness of genetic testing, in those who died of sudden cardiac death with a structurally normal heart.
Methods:
We performed an audit of those whose post-mortem reported a cause of death 'SADS' or 'unascertained'. Cases of 'SIDS' were excluded in this study.
Results:
In 27 cases of sudden cardiac deaths since the 01/01/11, there were 125 first-degree relatives. Of these 73 (58%) received an ECG and 53 (42%) received an echo. 21/27 tissues were genetically tested using the routine test panel of KCNQ1, KCNH2, KCNE1, KCNE2, SCN5A and RYR2. Pathogenic mutations were found in 2/21 of cases (SCN5A and RYR2 genes). In the majority of families where no gene mutation was identified, no diagnostic findings were found on clinical investigations. In addition, when these findings were combined with an earlier audit, we also found that in 22/56 (39%) cases, there was evidence in the post-mortem report of non-toxic levels of alcohol consumption around the time of death.
Conclusion:
The yield of pathogenic mutation (10%) was lower than reported literature (15-33%). This may be due to restriction of genes tested. Family follow up of those with a sudden cardiac death was adequate, although could be improved. This audit also highlights the prevalence of recent exposure to alcohol/non-toxic levels of alcohol at time of death in this group, and the role of alcohol in sudden death may merit further investigation.

PS05.81
Low yield of genetic testing for cardiac channelopathy in sudden unexplained infant death

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Introduction: Sudden unexplained infant death (SUDI, death under 12 months without cause on autopsy) accounts for 12.8% of infant deaths in Scotland. Cardiac channelopathy (CC) accounts for an estimated 5% of SUDI. Genetic variant identification in SUDI may have implications for recurrence risk and cascade screening. We present our experiences of genetic testing for CC in SUDI.
Methods: Retrospective analysis of gene testing for CC in SUDI at a single centre over 10 years. Six genes (SCN5A, KCNE1, KCNE2, KCNH2, KCNQ1, RYR2) were screened in all cases.
Results: In total, 24 cases of SUDI were referred; 3 were excluded due to alternative causes of death. Variants were detected in 7 (33.3%); none were conclusively pathogenic. Potential pathogenicity was assigned in two cases, though these variants are present in over 0.1% of 6,500 "normal" individuals in the NHLBI Exome Sequencing Project dataset. First-degree relatives were screened in both cases and no cardiac abnormalities were identified.
Discussion: In this small series, genetic testing did not uncover CC as a cause of SUDI. In contrast, 2/17 cases of sudden unexplained death in 1-5 year olds tested at our centre had pathogenic variants. There are clear difficulties interpreting variants due to conflicting literature evidence and minimal phenotypic data in most SUDI cases. CC is likely a rare cause of SUDI. Next generation sequencing panels may further increase interpretation complexity where variants are detected in little-studied genes. Cardiological investigation of first-degree relatives is essential when counselling families, particularly where variants of unknown significance are identified.

PM05.82

Telomere length and coronary heart disease: a bi-directional Mendelian randomization study

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Objective: To assess the bi-directional causal relationships between leukocyte telomere length (TL) and coronary heart disease (CHD) using a Mendelian randomization (MR) approach.**Methods:** Using a genetic risk score (GRS) of seven genetic variants associated with TL (Codd *et al*, 2013) as instrumental variable, we tested whether TL may be causally associated with CHD in two Swedish and one US cohort and, moreover, in summarized genome-wide association study (GWAS) data. Likewise, we examined if CHD may be causally associated with TL using a CHD GRS comprising forty-five variants (CARDIoGRAMplusC4D, 2013) as instrumental variable. **Results:** In observational analysis in our cohorts, we found shorter TL associated with higher risk of CHD incidence [hazard ratio (HR):1.20, 95% confidence interval (CI):1.04, 1.37] and a past CHD event associated with shorter TL (β:-0.07, 95% CI:-0.02, -0.12). TL GRS was associated with TL (β:-0.05, 95%CI:-0.02,-0.08), although its association with ever CHD was not significant [odds ratio (OR):1.01, 95% CI: 0.96, 1.06], nor was the MR analysis (OR: 1.29, 95%CI: 0.49, 3.42). However, the MR estimate from summarized GWAS data indicated causality (OR: 1.26, 95%CI: 1.12, 1.42). We further confirmed a causal effect of a past CHD on TL from summarized GWAS data (β:-0.04, 95%CI: -0.003,-0.08), but not in our own cohorts. **Conclusions:** Significant bi-directional associations between TL and CHD were observed in three cohorts of European ancestry. We further provided evidence for a causal effect of shorter TL on higher risk of CHD and for CHD event on shorter TL from summarized GWAS data.

PS05.83

Genetic determinants of the platelet glycoproteins. Results from the GAIT 2 Project.

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Introduction: Platelets play a key role in arterial and venous thrombosis. The glycoproteins (GP) on the platelet surface are involved in the adhesion process, by GP Ib-V-IX complex (CD42a and CD42b) and GP IV (CD36). They are also involved in the aggregation process, by GP IIb-IIIa complex (CD41 and CD61). Therefore, their involvement in thrombosis is very important. Previous studies have identified the genetic *loci* that code for the GP, but the phenotypic variance among individuals due to genetic differences have not been defined.
Materials and Methods: 935 individuals were studied from 35 large Spanish families recruited in the second phase of the GAIT (Genetic Analysis of Idiopathic Thrombophilia) Project. The GP were obtained using platelet-specific immunolabelling and flow cytometry. The fluorescence mean (F) and counts of the positive GP events percentatge (CT) were determined for the CD36, CD41, CD61, CD42a and CD42b. A variance component analysis based on maximal likelihood was applied to quantify the covariates effect, shared environment and genetic factors. Genetic effects were defined as heritability (h^2) which estimates the proportion of the total variability due to genetic factors.
Results: All of the GP parameters had high h^2 (range 0.37-0.71) with strong statistical significance (p-value range $4.48{\cdot}10^{-16}$-$7.44{\cdot}10^{-31}$). CD36CT and CD36F had the highest h^2 (0.71 and 0.61 respectively).
Conclusions: The high heritability of these traits means that genes play a pivotal role in determining the GP density on the platelet surface. Thus, it should be possible to identify specific genes that affect these platelet traits.
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PM05.84

A common polymorphism in the promoter of the Tumor Necrosis Factor-α (TNFA) gene mediates postprandial triacylglycerol following sequential meal ingestion

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Tumor necrosis factor-alpha (TNFA) is a proinflammatory cytokine produced from activated macrophages and has been shown to be associated with altered lipid metabolism. Dietary fat plays an important role in inflammatory response, with the level of TNFA reported to be increased following the ingestion of a high-fat meal. However, not all studies have found an association between TNFA and postprandial triacylglycerol concentration (TAG), which could be due to the variations in the *TNFA* gene or meal fat composition. The present study investigated the effect of a common promoter polymorphism, -308G→A (rs1800629), on circulating lipid metabolites, glucose and insulin concentrations over 8 h following sequential high-fat meals in 207 participants (BMI: 25.9±0.2 kg/m^2; age: 52±1 years). Biochemical measurements were determined at baseline and hourly interval following the test breakfast. At the baseline, the GG homozygotes were associated with higher fasting glucose concentration than A-allele carriers (P<0.001). Following the mixed breakfast (49 g fat) and lunch (29 g fat), a significant association was observed between genotype and the incremental area under the time response curve (iAUC) for the postprandial TAG (P=0.03) with a 41% higher TAG iAUC in the GG homozygotes (n=139) (69% of this population) than A allele carriers (n=63), where the genotype explained 18% of such variation. However, the impact of genotype on postprandial TAG was evident only in men (P=0.04). Our findings confirm previous associations and suggest that the *TNFA* -308G→A promoter polymorphism could be an important genetic factor predisposing to the increased level of triglyceride.

PS05.85

Next generation sequencing for congenital vascular malformations

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Next generation sequencing (NGS) is shedding light on the genetic causes of vascular anomalies and making it possible to combine tests for similar but distinct diseases. Vascular malformations (VMs) are a heterogeneous group of congenital circulatory malformations, characterized by morpho-structural and/or functional defects of various nature, severity and extent. They may occur in any part of the body and any type of vessel (arteries, capillaries, veins and lymphatic vessels). Although most VMs are sporadic (simple or combined), syndromic and familial forms also exist with substantial clinical overlap between lesions, and each can mimic the others.
We designed a 25-gene NGS panel for VMs with Mendelian inheritance (autosomal recessive, autosomal dominant or paradominant). Analysing 128 patients with sporadic congenital VMs, we identified 40 variations in 33 patients (25%). To estimate their pathogenicity, we looked up variations in dbSNP137, compared their frequency using data from the Exome Variant Server, evaluated the effect of protein amino acid substitution by in silico analysis, and also investigated the family if possible. Our results show that many genes can cause a wide variety of syndromic and non-syndromic disorders, confirming that genetic testing by NGS is useful and effective even in sporadic cases. Since the etiopathogenesis of many vascular lesions seems similar to that of tumours, we are screening tissue samples for mutations. Identification of causative genes and the possibility of tracing somatic mutations in tissues could provide important information about disease-free areas, useful for planning surgery to reduce the possibility of relapses.

PS06.01

Clinical spectrum of ACAD9 mutations

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Acyl-Co dehydrogenase family, member 9 (ACAD9) mutations are a frequent, usually fatal cause of early-onset hypertrophic cardiomyopathy and mitochondrial respiratory chain complex I deficiency in early childhood
Here, we report 7 unrelated patients with mutations in ACAD9. Heart failure or cardiovascular collapsus were the consistent presenting symptom in 7/7 probands. Age at onset ranged from the neonatal period to the first few months-years of life and echocardiography consistently revealed severe hypertrophic cardiomyopathy. At the time of diagnosis, one patient presented with neonatal encephalopathy with white matter anomalies on brain MRI, whereas no extra cardiac involvement was clinically present in 6 patients. The variability in the severity of the disorder was wide. Three patients had severe and rapidly progressive evolution and died before 2 years of age. One

patient recovered spontaneously. 3 patients were proposed for heart transplantation as they had no other organ involvement at the time of diagnosis. Among them, two survived. Most importantly, the 3 living patients latter developed delayed-onset neurologic and muscular symptoms, namely cognitive impairment, seizures, muscle weakness and exercise intolerance. Other organ involvement included proximal tubulopathy, renal failure, secondary ovarian failure or optic atrophy. Yet, while 2 patients enjoyed an almost normal personal and professional adult life, one patient experienced some learning difficulties and required special schooling. This is to our knowledge the first example of successful heart transplantation in ACAD9 mutation. Yet, the decision to carry out organ transplantation remains difficult as delayed neurological symptoms may occur and worsen despite their absence before transplantation.

PM06.02

Novel mutations in mitochondrial aminoacyl-tRNA synthetase genes and their phenotypic expression

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Mitochondrial aminoacyl-tRNA synthetases encoded by nuclear aaRS2 genes are essential enzymes in the translation of genetic information from mitochondrial DNA to the oxidative phosphorylation system complexes. Recently reported pathological mutations identified in 15 of the aaRS2 genes have been associated with diverse clinical presentations, usually characterized by early-onset and autosomal recessive transmission. Encephalopathy is the most common manifestation. Other clinical features include myopathy with anaemia, cardiomyopathy, tubulopathy and hearing loss. A relatively tight genotype-phenotype correlation has been reported for most of these cases. We report 10 patients, from five unrelated families, with infantile-onset mitochondrial disorder (Nijmegen score >4) and two mutant alleles for EARS2 (p.R55H/p.G109R and p.R55H/p.P419L), FARS2 (p.V345A/p.R419C), PARS2 (p.I80T/p.P364R), and RARS2 (p.Q208*/p.M342I) genes identified by whole-exome sequencing. Among the nine various substitutions, only p.R55H was reported previously. Eight novel mutations segregated with the disease within the families. Moreover, their pathogenic role was determined by using five different prediction algorithms. The characteristic "neurological" phenotype, reported previously, was associated with EARS2, PARS2 and RARS2 defects, however, facial dysmorphism, failure to thrive and icterus revealed in one compound heterozygote for EARS2 mutations (p.R55H/p.P419L). In addition, our patient with Alpers syndrome due to PARS2 mutations confirms genotype-phenotype correlation observed so far in only one child. In the family with heterozygous variants in FARS2 a completely new phenotype, including cardiomyopathy and renal hyperkaliemic acidosis was observed.
In conclusion, novel mutations in four aaRS2 genes expand the list of aaRS2-associated diseases, including new phenotypes potentially related to mutations in FARS2 and EARS2 genes.
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PS06.03

A Comparative Assessment of Plasma Peptides, GDF-15 and FGF21, as Biomarkers for Mitochondrial Disease Among Two Biobank Cohorts

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Background: Recent studies have proposed that transforming growth factor-β superfamily member, GDF-15, and fibroblast growth factor 21, FGF21, might be useful biomarkers for mitochondrial disease. We hypothesized that we could verify the sensitivity and specificity of GDF-15 and FGF21 as plasma biomarkers from our Mitochondrial Disease Biobank of over 300 individuals with a clinical diagnosis or suspicion of mitochondrial disease as determined by clinical, molecular, enzymatic, and/or histological findings.
Methods: Thirty-three participants with confirmed primary mitochondrial disease (MELAS, MERRF, NARP, CPEO,and others) provided EDTA plasma specimens. Age-matched, control plasma for reference ranges were obtained from 120 Mayo Clinic Biobank donors, who did not have a clinical history of diabetes, cardiac, renal, or liver disease. Plasma GDF-15 and FGF21 were measured by ELISA and compared across cohorts.
Results: Mitochondrial disease participants demonstrated plasma GDF-15 (median: 1163 pg/mL; 1st-99th%-iles: 491-6817 pg/mL) and FGF21 (median: 486 pg/mL; 1st-99th%-iles: 211-2816 pg/mL) levels that were higher than the control cohort (GDF-15 median: 423 pg/mL; 1st-99th%-iles: 230-1560 pg/mL and FGF21 median: 394 pg/mL;1st-99th%-iles: 88-1658 pg/mL). Receiver operator characteristic curves of GDF-15 and FGF21 demonstrated that GDF-15 specificity was 88% while FGF21 was only 41%. In addition, participants with MELAS showed higher GDF-15 levels (median: 3290 pg/mL) than any other mitochondrial disease diagnosis (GDF-15 median: 921 pg/mL).
Conclusions: Among two promising biomarkers touting utility for mitochondrial disease, GDF-15 demonstrated better specificity and sensitivity than FGF21. Moreover, the elevations of GDF-15 highlights a putative integrative role for this signal transducer in the pathology of mitochondrial disease.

PM06.04

Gene duplication in congenital adrenal hyperplasia (CAH) complicates genetic counselling

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Congenital adrenal hyperplasia (CAH) is an autosomal recessive condition with an incidence of about 1 in 12,000 individuals. Over 90% of cases are due to mutations in the gene for 21-hydroxylase (CYP21A2) and ten mutations account for the majority of those known. The presentation of CAH, classical, simple virilising or non-classical, is related to mutation type.
In recent years it has been recognised that carrier detection can be complicated by rare haplotypes that contain duplicated copes of CYP21A2. Our Italian patient was tested as his partner had already been identified as a carrier for a mutation primarily associated with non-classical CAH. His result showed that he is heterozygous for a classical CAH mutation, p.(Gln319Ter) but also that he has at least 3 copies of the CYP21A2 gene. Gln319Ter is a common CAH mutation associated with a classical to non-classical phenotype depending on mutation combination. However, a rare European haplotype has been described with Gln319Ter in the context of a duplication. Individuals who have this haplotype are therefore not carriers as there is a functional gene in cis. This rare haplotype is in linkage disequilibrium with rare variants, in intron 2 and the 3' untranslated region of CYP21A2, as well as HLA-B50-Cw06 subtype, and has been reported in the Italian population. Accurate preconception, and extended family counselling, requires distinguishing between Gln319Ter in the context of a single CYP21A2 gene or the duplication haplotype, using family studies and additional markers, where possible.

PS06.05

A very common inborn error of carnitine biosynthesis about which little is known

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Introduction: *TMLHE* deficiency is a very common inborn error of carnitine biosynthesis. The gene is X-linked, and about 1 in 350 healthy males of European descent have a deletion of exon 2 resulting in inability to synthesize carnitine. *TMLHE* deficiency likely is a risk factor for autism, but the penetrance for autism is less than 5%, and replication studies are needed.
Materials and Methods: The frequency of exon 2 deletion was determined in 10,678 male samples undergoing array CGH for evaluation of heterogeneous disabilities and in 2124 male samples from the Baylor Mendelian Genome Center exome project. A case evaluation was performed on a *TMLHE* deficient male with autism and two episodes of regression.
Results: Deletion of exon 2 of *TMLHE* was found in 50 of 10,678 males undergoing array CGH (0.46%) compared to 7 in 2245 males undergoing exome sequencing in the Mendelian studies (0.31%). The phenotypic data for the deficient males are being tabulated. Carnitine deficiency in a *TMLHE* case suggests that deficits in carnitine biosynthesis may be responsible for some cases of regression in individuals with autism.
Conclusions: Deletion of exon 2 is confirmed to be very common in various clinical samples. A case study suggests that carnitine supplementation may be useful in treating regressive autism episodes in patients with *TMLHE* deficiency. It is unknown if infant males with *TMLHE* deficiency should receive any dietary modification. It is unknown if there are genetic, dietary, or microbiome factors that influence the development of autism in *TMLHE* deficient males.

PM06.06

A novel nonsense mutation of the ABHD5 gene causes the early onset of Chanarin-Dorfman syndrome

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Chanarin-Dorfman Syndrome (CDS), also known as Neutral Lipid Storage Disease with Ichthyosis (NLSD-I), is a rare autosomal recessive lipid storage disease. This syndrome is characterized by non-bullous congenital ichthyosiform eryhthroderma (NCIE), hepatomegaly and liver steatosis. Additional clinical features include muscle weakness, ataxia and sometimes neurosensory hearing loss, subcapsular cataracts, nystagmus, strabismus and mental retardation. Patients are often born as collodion babies. ABHD5 gene mutations have been identified as the cause of CDS. This gene codifies for the α/β-hydrolase domain-containing protein 5 (ABHD5), a co-activator of the patatin-like phospholipase domain-containing protein 2 (PNPLA2). PNPLA2 is a lipase, associated to the lipid droplets surface, that catalyzes the initial step of triacylglycerols lipolysis. Until now, about 40 different mutations of ABHD5 gene are associated with CDS onset and 15 of them result in truncated proteins.
In a 29-month-old Indian girl we have identified a homozygous nonsense mutation (c.297C>A) of ABHD5 gene, causing the production of a short truncated protein (p.C99X). The little CDS patient presented NCIE, hepatomegaly, diffuse hepatic steatosis and splenomegaly. After the molecular confirmation of CDS diagnosis, she was put on a low fat diet.
Our results show that the early onset of CDS (in Indian patient) is probably due to the production of an ABHD5 protein which loses the putative interacting domain required for PNPLA2 activation. Moreover, an early initiation of a diet poor in long chain fatty acids might improve the liver condition and prevent severe systemic damages.

PM06.08

New genetic insights into the spectrum of disorders of glycosylation: a patient with multiple congenital anomalies

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Introduction: Glycosylation is essential for human development. More than 100 clinically diverse inherited disorders are known to result from glycosylation defects. One subtype of the congenital disorders of glycosylation (CDG) is caused by defects in glycosaminoglycan (GAG) synthesis. Here, we describe an infant from a consanguineous family with a GAG-CDG. Phenotypic features include respiratory insufficiency, discrete skeletal anomalies, renal insufficiency, and T-cell depletion.
Methods: We performed whole-exome sequencing (WES) on patient leucocyte DNA to identify the causative mutation. Immunocytochemistry was conducted to characterize the identified mutations functionally in patient-derived and control fibroblasts. Different GAG levels were measured in fibroblasts and urine from the patient and several controls.
Results: Through WES we detected two homozygous missense mutations in highly conserved regions of two glycosylation genes regulating GAG synthesis and modification. It remains unclear whether mutations in one or both of the genes contribute to the clinical phenotype. We found that one of the mutated proteins that normally localizes to the Golgi apparatus was absent in this organelle in patient cells. Immunocytochemical analysis of the other mutated protein failed. Finally, heparan sulphate GAG levels were significantly reduced in patient-derived fibroblasts, though within the normal range in urine.
Conclusion: We describe an infant with multiple congenital anomalies. WES analysis revealed two mutations in two glycosylation genes. One of the mutated proteins mislocalizes in patient-derived fibroblasts. Our data indicate that this patient has a GAG-CDG.
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PS06.09

Distribution of nine most common CYP21A2 point mutations in Macedonian patients with congenital adrenal hyperplasia

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Background: Nine pseudogene-derived point mutations account for about 80% of all defects in the CYP21A2 gene coding the 21-hydroxylase enzyme that deficiency is present in 90-95% of all cases with congenital adrenal hyperplasia (CAH). It can present as severe salt wasting (SW) or simple virilizing (SV) form, or the milder late onset form (LO).
Methods: Using the PCR/ACRS method, we have studied nine CYP21A2 point mutations in 66 Macedonian patients with clinical and laboratory signs of CAH evaluated at Department of Endocrinology and Genetics, University Children's Clinic, Skopje, Republic of Macedonia. Of the CAH patients 25 had SW form, 17 the SV and 24 the LO form of the disease.
Results: Six different mutations were detected in 72.7% alleles of the Macedonian patients. The most prevalent mutation was IVS2, present in 44 alleles (33.3%), followed by the P30L in 26 (19.7%), Q318X in 15 (11.4%), I172N in 6 (4.5%), V281L in 5 (3.8%) and R356W in 3 alleles (2.3%). Del 8ntG110, cluster exon 6 and InsT307 were not found. Mutations were detected in 86% of the SW, 85.3% SV and 50% LO alleles. In 60.6% (40/66) of the Macedonian patients complete genotype was revealed (31 homozygous and 9 compound heterozygous) with good correlation with phenotype. The most common genotype was IVS2/IVS2 (33.9%). Sixteen (24.2%) patients were heterozygotes and 10 (15.2%) harboured none of the tested mutations.
Conclusion: We observed high P30L and Q318X frequencies and low I172N frequency in our population compared to the most of the other European countries.

PM06.10

Subjects treated with migalastat demonstrate stable renal function, reduced left ventricular mass and gastrointestinal symptom improvement in Phase 3 and a long-term extension study of Fabry Disease

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Objectives: Migalastat (1-deoxygalactonojirimycin, AT1001) is an orally-administered investigational pharmacological chaperone for Fabry disease that selectively binds and stabilizes α-Gal A, leading to increased lysosomal activity. Study 011 (AT1001-011, NCT00925301) included a 6-month double-blind placebo-controlled period (Stage 1) and an 18 month open-label extension. Sixty-seven subjects were randomized; 48 subjects completed the study and continued in long term extension Study AT1001-041.
Methods: Estimated GFR (eGFR) was assessed every 3-6 months using the CKD-EPI and MDRD equations. Left ventricular mass index (LVMi) was assessed by echocardiography every 6-12 months by a blinded central laboratory. Gastrointestinal symptoms were assessed every 6 months using the Gastrointestinal Symptom Rating Scale (GSRS). p-values are unadjusted for multiple comparisons. Efficacy results are reported for the 50 subjects with amenable *GLA* mutations.
Results: eGFR remained stable in subjects treated up to 48 months, with mean annualized eGFR changes (±SEM) of -0.80±0.60 (CKD-EPI) and +0.70±0.80 (MDRD) mL/min/1.73m^2/yr. LVMi was significantly reduced in subjects treated up to 48 months (-8.0 ±5.5 g/m^2); the largest reductions were observed in subjects with abnormal LVMi at baseline (-17.2±16.5 g/m^2). Improvement was observed in the diarrhea domain of GSRS during the 6-month double-blind period (-0.3 migalastat, +0.2 placebo, p=0.03), and in diarrhea, indigestion and reflux domains during the open-label extension.
Conclusions: Treatment with migalastat for up to 48 months was associated with stable renal function, reduced LVMi and improved gastrointestinal symptoms in patients with Fabry disease who have amenable GLA mutations.

PS06.11

DNA analysis of familial hypercholesterolemia in Slovakia

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Introduction: The clinical phenotype of familial hypercholesterolemia (FH) is associated with mutations in genes encoding LDL receptor, apolipoprotein B and PCSK9. The general prevalence of FH in most countries is 1:500,

that accounts for approximately 10 000 patients in Slovakia. However, spectrum of mutations in Slovak patients has not been published previously. The aim was to analyze spectrum of mutations in *APOB, LDLR* and *PCSK9* genes in patients with clinical suspicion of FH.
Materials and Methods: The p.Arg3527Gln mutation in *APOB* gene was tested by real-time PCR. The mutations in *LDLR* and *PCSK9* genes were analyzed by Sanger sequencing. The large rearrangements in *LDLR* were assessed by MLPA analysis.
Results: Out of 136 probands and 103 relatives, we have confirmed the presence of *APOB* mutation p.Arg3527Gln in 9 probands and 2 relatives, respectively. In remaining 127 probands and 101 relatives, the presence of pathogenic mutation in *LDLR* was confirmed in 58 probands and 49 relatives. All together, we have identified 41 mutations and 9 new variants in *LDLR* gene. Their potential pathogenicity predicted by *in silico* analysis was confirmed by cosegregation analysis in given families. Direct sequencing of *PCSK9* gene revealed no pathogenic mutation.
Conclusions: By DNA diagnostics, the clinical diagnosis of FH was confirmed in 49.3% probands and 49.5% of relatives. The high incidence of mutations in the genes analysed in our study emphasise the importance of genetic testing for targeted treatment and prevention in families with FH in Slovakia.
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PM06.12
Mutation spectrum in German patients with familial hypercholesterolemia

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Autosomal-dominant familial hypercholesterolemia (ADH) is characterized by elevated plasma levels of low-density lipoprotein cholesterol (LDL-C) and a dramatically increased risk to develop cardiovascular disease (CVD). The prevalence of ADH is about 1:500, with a higher frequency because of founder effects in some populations. Mutations in three major genes have been associated with ADH: LDL receptor gene (LDLR), apolipoprotein B gene (APOB) and proproteinconvertase subtilisin/kexin 9 gene (PCSK9).
We investigated the mutation spectrum in 120 patients (51% females) clinically diagnosed with possible or probable ADH. We sequenced the coding region of the LDLR gene followed by sequencing the site of the major disease causing mutation in the APOB gene, c.10580G>A (p.Arg3527Gln) and, finally, sequencing of the PCSK9 coding region,.
Pathogenic mutations were identified in 53 patients (44%) in one of the analysed genes. As expected, most of the mutations were identified within the LDLR gene (about 90%). Heterozygous missense mutations in the APOB gene were detected five patients and no mutation in the PCSK9 gene was found.
In 48 patients we detected a total of 51 LDLR mutations. 33 of the patients showed missense mutations. In addition we identified 6 small deletions (≤20 nucleotides), 5 slice-site mutations and 7 nonsense mutations. Three of the 48 patients were probable compound heterozygous. Six of the mutations identified have not been described before.
The results of the mutation screening will be presented together with phenotypic data and will be discussed with respect to previous data on German ADH mutations and phenotype-genotype correlations.

PS06.13
Acid ceramidase deficiency: clinical implications of an emerging spectrum and potential therapies

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Mutations in the ASAH1 gene lead to acid ceramidase deficiency, the resultant accumulation of the lipid ceramide, and a distinct variety of disease phenotypes, culminating in two recognized diseases: Farber disease and Spinal Muscular Atrophy with Progressive Myoclonic Epilepsy (SMA-PME). Farber disease represents a broad clinical spectrum presenting from infancy through late childhood, associated with the pro-inflammatory and pro-apoptotic characteristics of ceramide. SMA-PME, a late childhood onset, primarily neurologic disease, has been less well characterized to date, and the pathophysiologic mechanisms underlying the clinical manifestations are the subject of ongoing study. The prevalence of both diseases is currently unknown and awareness of them is limited due to their rarity; both are likely underdiagnosed.
We have collected data on over 20 living Farber patients, the largest cohort to date, including data on biochemical and immunologic phenotypes. Our findings reinforce the validity of the characteristic symptoms of Farber disease: early-onset polyarticular arthritis, subcutaneous nodules and dysphonia. However, it also reveals that there are patients who present with only one or two of these symptoms, and that the spectrum of disease includes remarkably attenuated forms with relatively little associated disability. We feel there is an indication for diligent screening of certain pediatric (and even young adult) polyarticular arthritis patients for Farber disease, and certain therapy-resistant epilepsy patients for SMA-PME. Such screening is being initiated and natural history studies are planned in both diseases.
Recombinant human acid ceramidase enzyme therapy is currently under development and is expected to enter clinical trials in 2016.

PM06.14
Development of a cell-based approach to identify small molecules as regulators of FGF23 signalling

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Fibroblast growth factor 23 (FGF23) is a key regulator of phosphate homeostasis. It plays a critical role in hereditary and acquired hypo- and hyperphosphatemic disorders. Moreover, FGF23 has emerged as a promising biomarker for the prediction of adverse clinical outcomes in patients with chronic kidney disease (CKD), since it might be related to mortality, cardiovascular abnormalities and disease progression. FGF23 is a bone-derived endocrine factor that regulates renal tubular phosphate reabsorption and vitamin D metabolism by activating FGF receptor (FGFR)/Klotho complexes in the kidney. Mitogen-activated protein kinase (MAPK) pathway is employed as a major signalling pathway. To investigate the molecular mechanisms underlying FGF23 actions in more detail, we established a cell model of FGF23-inducible cells stably expressing Klotho (HEK293-KL cells). The FGF23-mediated induction of HEK293-KL cells was shown by detecting the activation of MAPK pathway, which could be reduced by the use of two known small-molecule inhibitors of this pathway: SU5402 and U0126. Based on our established cell model, reporter assays suited for high-throughput screening (HTS) were developed to identify novel small-molecule compounds that modulate FGF23 signalling. In a pilot screen, robustness and appropriateness of the method could be verified. The discovery of candidate hits would provide the basis to manipulate FGF23 signalling and would potentially validate this pathway as druggable in disorders caused by altered actions of FGF23.

PS06.15
Four cases of gangliosidosis GM1 with prenatal onset in a consanguineous family

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We present four cases of gangliosidosis GM1 in a consanguineous family from gipsy minority. The first case is a 6 months girl (born in 2007) with coarse facies, periorbital oedema, broad nasal bridge, bulbous nose, short neck, hepatosplenomegaly, muscular hypotonia, psycho-motor disability. The girl died at 11 months. Second case (I degree cousin with first case) is a 3 weeks girl (born in 2008) with delayed weight and height development, coarse facies, periorbital oedema, broad nasal bridge, bulbous nose, macroglosia, retrognathia, low set years, short neck, sacral dimple, thymus hypertrophy, cardiomegaly, hepatomegaly, splenomegaly, muscular hypotonia and psycho-motor disability. This girl died at 10 months. The last couple have a normal girl and other two affected children a boy born in 2012 and girl born in 2015. Both were diagnosed in neonatal period, after pregnancies with ascites and hepatomegaly. Both presented: coarse facies, generalised oedema, broad nasal bridge, bulbous nose, macroglosia, retrognathia, low set years, short neck, cardiomegaly, hepatosplenomegaly, muscular hypotonia. The boy presented a huge scrotal oedema. In the 4th child of couple were found corpuscles Alder-Reilley and a 0,004 nmol/spot level of -galactasidasis. The baby died at age of 8 months. In all cases the genetic analysis was impossible. We made the genetic counselling, but for religious and low level

of education reasons, the couple don't take in account the risk. We consider that in this family could be useful to identify the mutation in the last affected child and search the same mutation in other risk's individuals.

PM06.16

Delineation of a poorly studied type of bone crisis in patients with type 1 Gaucher disease: bone crises of the small bones of the hands and feet

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Background: Type 1 Gaucher disease is the most common form of Gaucher disease, an inherited lysosomal storage disorder affecting around one in every 40,000-60,000 people in the general population and approximately one in every 800 people of Ashkenazi Jewish origin. Bone crises in type 1 Gaucher disease are reported in long bones, in weight bearing bones and other bones, but rarely in the small bones of the hands and feet.
Method: We retrospectively examined the incidence of bone crises, including small bone crises, in patients followed at the Rabin Medical Centre, Petah Tikva, Israel, before and following the initiation of imiglucerase enzyme replacement therapy (ERT).
Results: Of 100 type I Gaucher disease patients, 30% experienced one or more bone crises. Small bone crises represented 31.5% of all bone crises and were preceded by crises in other bones. While the incidence of long bone crises decreased after the initiation of ERT, the incidence of small bone crises increased. Almost 60% of all patients with bone crises were of the N370S/84GG (representing only 30% of the cohort) suggesting a greater susceptibility of N370S/84GG patients to severe bone complications. These patients also underwent the greatest number of splenectomies (70.6% of all splenectomised patients) consistent with a more severe disease phenotype. Patients who underwent splenectomy showed a trend towards increased bone crises after surgery. **Conclusion**: Physicians should be aware of the possibility of bone crises in the hands and feet and should consider imaging studies to investigate unexplained pain in the hands and feet.

PS06.17

Association of PTPN22 gene functional variant C1858T, HLA-DQ alleles and autoantibodies with Type-1 Diabetes Mellitus in Kuwaiti children

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An interplay between susceptibility genes, immune mediators and environmental factors predispose susceptible individuals to T1DM. We have determined the prevalence of PTPN22 gene C1858T functional variant, HLA-DQ alleles and three autoantibodies in Kuwaiti children with T1DM. This study included 191 Kuwaiti children with TIDM and 101 controls (healthy, ethnically matched). The diagnosis of T1DM was based on the ISPAD criteria. The genotypes for PTPN22 gene variant C1858T (R620W) were identified by PCR-RFLP. HLA-DQ alleles were determined by sequence-specific PCR in 178 patients. The presence of autoantibodies (ICA, INS and GAD) were determined by radioimmunoassay. The variant genotype of the PTPN22 gene was detected in homozygous/heterozygous combination in 39% patients compared to 27% in controls. The homozygous TT-genotype was detected in 8% patients compared to 0.99% in controls ($p < 0.001$). Nine different combinations of HLA-DQ alleles were detected in patients. In 55% patients, the genotype was either homozygous for DQ2 or in combination with a DQ8 allele. In 36% patients, the genotype was homozygous DQ8 or with other alleles. Collectively, 91% of the patients had either DQ2 or DQ8 alleles. In patients with TT-genotype of PTPN22 gene, 93% had at least one DQ2 allele and 60% carried either a DQ2 or a DQ8 allele. In T1DM patients with TT-genotype, GAD autoantibody was detected in 83%, INS-Ab in 67% and ICA-Ab in 54% cases respectively. Our data demonstrate that the variant T-allele of PTPN22 gene and HLA-DQ2/DQ8 alleles constitute significant determinants of genetic predisposition to T1DM in Kuwaiti Arabs.

PM06.18

International datasharing of Exome sequencing results for the clinical delineation of extremely rare disorders: example of autosomal recessive mutations in GFER

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Introduction: A family with 2 affected sibs presenting with congenital cataract, progressive muscular hypotonia, hypotrophy, and psychomotor delay followed by severe neurological regression associated with seizures was followed up for several years. The oldest sibling died at age 21 years, and her brother displays severe encephalopathy with cachexia. Along this diagnostic odyssey, a mitochondrial condition was suspected after morphological analysis of a muscle biopsy, exhibiting rare COX-negative fibers. Whole exome sequencing (WES) detected autosomal recessive truncating mutations in *GFER*. This gene has already been assigned in human pathology in three siblings born to healthy consanguineous Moroccan parents (Di Fonzo et al., 2009). The siblings carried a homozygous missense mutation in *GFER*, and presented a very similar phenotype including congenital cataract, progressive muscular hypotonia, sensorineural hearing loss, developmental delay, scattered COX-negative muscle fibres with moderate reduction of complex IV activity.
Material and Methods: Through an international data sharing, another family with two siblings has also been identified, also presenting with bilateral cataracts, global developmental delay, lactic acidosis, and increased mitochondrial DNA copy number. Similarly, the diagnostic approach in this family was WES.
Results: These 7 cases issued from 3 families strongly support the existence of a new recognizable mitochondrial disorder associating progressive encephalo-myopathy with congenital cataract secundary to autosomal recessive mutations in the *GFER* gene.
Conclusions: This report highlights the clinical utility of WES in rare diseases and underlines the importance of a broad data sharing for an accurate interpretation of previously unrecognised clinical entities.

PS06.19

Molecular Investigation of Glutaric Aciduria Type1 in Iran

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Glutaric Acidemia, Type I (GA I), was first described in 1975. The disease is caused by a genetic deficiency of the enzyme, Glutaryl-CoA Dehydrogenase (GCD), which leads to the buildup of Glutaric acid in the tissues and its excretion in the urine of affected patients. GA I is one of the most common organic acidemias and has an estimated incidence of about 1 in 50,000 live births. Because of the initial slow progression of clinical symptoms, GA I is frequently undiagnosed until an acute metabolic crisis occurs A total of 25 unrelated patients suspected to GA1 were investigated in our study. Genomic DNA was extracted from peripheral blood cells of the 25 probands whom were biochemically and/or clinically and/or neuro-radiologically suspected to GA1. 15 of them had elevated glutaric acid in the urine organic acid test. PCR and direct sequencing of all 11 exons and their flanking region of the GCDH gene were examined.Some of them were investigated for known mutation in the other therir family members..Fifteen patients had homozygous mutations and 10 patients were normal for GCDH gene. Our Results Showed:• 60% Known mutation were found in our 15 patients• 80% can be detected by 4 exons sequencing so for molecular investigatins exon 6, 7, 8, 10 are good choice for beginning of analysis• 33% was mutation in exon 7, so because of the cost of genetic diagnosis we suggest that investigation begin with this exon.• Pro 348 Leu was most detected 20%.• 40% are new mutations wich will be investigated for phenotype Genotype Correlations

PM06.20

GENETIC ANALYSIS OF GLYCOGEN STORAGE DISORDERS BY MASSIVE PARALLEL SEQUENCING

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Glycogen storage disease (GSD) is a group of genetic disorders resulting from an abnormal metabolism of glycogen. Currently 22 different types of GSD are known. The nonspecific clinical picture and the lack of specific biomarkers hamper diagnosis being DNA methods the gold standard for successful diagnosis. Nevertheless the cost and time consuming gene by gene Sanger sequencing has made difficult to achieve successful diagnosis and many GSD patients in our country remain without definitive diagnosis. Here, we report the genetic analysis in a cohort of 31 Spanish patients referred to our laboratory. We have diagnosed 12 patients by conventional Sanger sequencing of genes associated with the clinical history and in the last year, 15 by massive parallel sequencing with a customized panel or clinical exome sequencing. Overall GSD type III (AGL gene) and GSD IXa (PHKA2 gene) were the most frequent GSD deficiencies accounting for close to 75% of the GSD patients. The mutational spectrum includes 29 different mutations, 14 novel, most of them loss-of-function mutations. By clinical exome sequencing analysis we have diagnosed four further patient with mutations, ALDOB, LIPA, NKX2-5 and CPT2, not related to GSD but with shared phenotypic characteristics such as liver, muscular and cardiac dysfunction. This study shows that next generation sequencing is an accurate high throughput method for the genetic diagnosis of Glycogen Storage Disease and other related clinical diseases of genetic origin enabling appropriate therapeutic and genetic counselling.

PS06.21
Homozygosity mapping using SNP microarray as a useful diagnostic tool in consanguineous populations

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Introduction: Consanguinity increases the coefficient of inbreeding and the likelihood of the presence of pathogenic mutations in homozygotic state. An SNP-based microarray is a useful tool not only for studying copy number variants but also for the detection of uniparental disomy and regions of homozygosity (ROH) throughout the genome. Evaluation of ROH can guide medical geneticists to focus on a single specific gene in order to achieve molecular diagnosis in families with common parental ethnic background or consanguinity. In addition, ROH analysis can eliminate genes as possible candidates in highly heterogeneous recessive conditions.
Methods: We describe six cases in which analysis of SNP-based microarrays (Illumina HumanOmniExpress-24 v1.0 BeadChip) has assisted us reaching molecular diagnosis in consanguineous families by sequencing a single candidate gene that was localized within homozygosity region.
Results: We identified a disease-causing mutation in all the families. All the genes are related to metabolic or neurological disorders and include: CRLF1 (Crisponi syndrome/CISS1 syndrome), PHKG2 (Glycogen storage disease IXc) NPC1 (Niemann-Pick C disease), MPV17 (hepatocerebral mitochondrial DNA depletion), MAN2B1 (Alpha-mannosidosis), and MOCS2 (Molybdenum cofactor deficiency).
Conclusions: These examples demonstrate the great diagnostic value of SNP microarray in deciphering molecular defect in consanguineous families by minimizing the need for massive parallel sequencing and reducing the cost of the molecular work-up.

PM06.22
Impaired mitochondrial RNA processing in HSD10 disease

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Introduction: Missense mutations in the 17ß-Hydroxysteroid dehydrogenase type 10 gene (*HSD17B10*) result in HSD10 disease, a rare X-chromosomal childhood disorder mainly characterized by psychomotor regression and cardiomyopathy. HSD10 is part of the mitochondrial RNase P enzyme complex required for 5`-end cleavage of mitochondrial precursor tRNA´s. Here, we analyzed whether *HSD17B10* mutations are linked to RNase P deficiency.
Methods: Expression of RNase P proteins was analyzed by Western blots. Real-time RT-PCR and Northern blot analysis were performed to quantify mt-tRNA processing in HSD10 knock-down cells, patient (R130C and Q165H) and control fibroblasts.
Results: HSD10 protein expression was strongly reduced in fibroblasts with mutation p.R130C which was associated with low expression of MRPP1 protein, an essential component of the RNase P complex. Knock-down experiments further revealed that expression of HSD10 is necessary for normal MRPP1 expression. Mutation p.Q165H shows equal amounts of HSD10 and MRPP1 protein than controls. Transcript analysis demonstrated that reduced HSD10/MRPP1 expression resulted in an accumulation of precursor transcripts of mitochondrial heavy strand RNA. Furthermore, in fibroblasts with mutation p.Q165H mitochondrial RNA processing was equal than in controls. This finding correlates well with the observed phenotype in patients with mutation p.Q165H who exhibit normal development. Interestingly, processing of light strand transcripts was not affected in patient cells.
Conclusion: Our results indicate that HSD10 plays a crucial role in the HSD10/MRPP1 complex formation, and loss of HSD10 results in impaired mitochondrial tRNA processing of heavy strand transcripts. The findings provide a novel explanation for mitochondrial dysfunction observed in HSD10 disease.

PS06.23
Pharmacogenetics in channelopathies causing congenital hyperinsulinism in Slovakia

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Congenital hyperinsulinism (CHI) is the most common cause of the persistent hypoglycaemia in children. Mutations in KCNJ11 and ABCC8 genes coding potassium channel subunits are major cause of CHI development in newborns. The type of B-cell hyperplasia (focal or diffuse) depends on the type of mutation and determines diagnostics, treatment and further prognosis. This study aimed to evaluate genetic cause of severe hypoglycaemia and recommend an appropriate therapeutic approach in particular cases.
Patients and Methods: For genetic testing 14 unrelated probands with congenital hyperinsulinism were reffered over the ten years (2005 - 2014) throughout Slovakia. For molecular diagnosis of the disease, direct sequencing of ABCC8 and KCNJ11 genes was carried out.
Results: We found mutations in ABCC8 or KCNJ11 in 36% (5/14) of patients. We identified two paternaly inherited ABCC8 mutations (Q444H and c.2694+1G>C) in two diazoxide-unresponsive patients with focal form of CHI. Subsequently, both patients underwent pancreatic surgery to reduce B-cell mass. Another two paternaly inherited mutations were found in two diazoxide sensitive patients, one novel in KCNJ11 (T180N) and another known in ABCC8 gene (V17A). Compound heterozygote KCNJ11 mutations (Q52*, R30G) were identified in a 4-months old boy with severe diazoxide-unresponsive hypoglycaemia. Based on this results treatment with octreotide was started. Finally, the combination of octreotid with frequent feedings through a gastrostomy led to normalization of glycaemia.
Conclusion: Taken together, we have resolved etiology in 36% CHI cases, which helped to choose the most appropriate therapeutic procedure.
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PM06.24
Dual genome investigation in Leber Hereditary Optic Neuropathy

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Leber hereditary optic neuropathy (LHON) is a maternally inherited mitochondrial disorder characterized by retinal ganglion cell degeneration leading to acute bilateral loss of central vision, particularly affecting young adult males. LHON has a negative detrimental impact for the quality of life and it is by far the most common mitochondrial genetic OXPHOS disease.
According to literature, the majority of LHON patients with mitochondrial DNA (mtDNA) mutations harbor one of three primary point mutations (90-95%): m.3460G>A, m.11778G>A and m.14484T>C.
A sample of 10 patients suspected of LHON, followed at the Ophthalmology and Neurology Departments of CHUC, was studied (2 females and 8 males: age mean±SD: 35±15 years). Total DNA was extracted from peripheral blood and muscle. Analysis of mtDNA and 513 nuclear genes related to mitochondrial disorders was performed by next generation sequencing (NGS).
The typical LHON mtDNA mutations were found in 5 patients: 3 with m.11778G>A, 1 having m.14484T>C and 1 with m.3460G>A.
The nuclear DNA (nDNA) analysis revealed that 8 samples have alterations, all heterozygous, with a predictable functional impact, according to in silico

analysis; the majority of these alterations (9) are novel. All affected genes are related to mitochondria network reinforcing the communication between the two genomes, but this issue must be further investigated.
This study was financed by Foundation for Science and Technology (PTDC/DTP-EPI/0929/2012 and PEst-C/SAU/LA0001/2013-2014).

PS06.25

LHON/MELAS overlap syndrome in a girl with complex I deficiency caused by very rare mtDNA mutation m.13046T>C

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LHON and MELAS syndrome are mitochondrially-inherited diseases characterized by subacute visual failure and variable multisystemic presentation, respectively. We present a unique case of LHON/MELAS overlap syndrome in a 13-year-old girl with genetic background previously described in one patient with MELAS/Leigh syndrome.
Results: A firstborn girl of healthy, unrelated Caucasian parents was born after uneventful pregnancy and delivery. Patient´s mother and two younger brothers are clinically healthy, but the mother suffered from two misscarriages. The disease manifested at the age of 12 years with an abrupt, painless and simultaneous loss of vision with a subsequent stabilization two months later. The ophtalmologic examination documented visual acuity of 0.6/20 (O.S.) and 4/20 (O.D); the perimetry objectified a bilateral caecocentral scotomas. Over the next months, the girl developed a moderate sensorineural hearing loss, vertigo, headache, anhedonia and thyroiditis. There was no Leigh syndrome or postictal changes on brain MRI. Initial genetic analyses excluded all three LHON prevalent mutations in mtDNA. Extensive metabolic workup documented elevated CSF lactate of 4.25 mmol/l (C in ND5 subunit (urine 71%, muscle 70%, hair follicles 44%, fibroblasts 40%, buccal smear 34% and blood 27%). Surprisingly, the mutation was not detected neither in patient´s mother nor her two younger brothers.
Discussion: We report the second known patient with m.13046T>C mutation. Based on a combination of clinical symptoms, we concluded the condition as LHON/MELAS overlap syndrome. Institutional support was provided by IGA NT 14156/3, IGA NT 13114/4, RVO-VFN 64165/2012, GAUK 38515/2015.

PM06.26

Solute carrier family 19 (folate transporter) member-1 defeat leads to lipid accumulation in hepatocytes

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The etiology of nonalcoholic fatty liver disease (NAFLD) remains poorly understood. Several molecular mechanisms have been proposed to explain how steatosis progresses to nonalcoholic steatohepatitis and hepatocellular carcinoma. One of them is altered methionine metabolism, which is coupled to the folate biosynthetic pathway.
Herein, we report a multicenter association study of 756 biopsy-proven NAFLD patients and controls, which together with various functional approaches allowed us to describe the role of the *solute carrier family 19 (folate transporter) member 1* (*SLC19A1*) gene in the development of NAFLD.
Two (one missense) out of the three studied SNPs within *SLC19A1* showed a significant association ($p<10^{-3}$) with NAFLD for the single-marker allelic test. In addition, minor homozygous genotypes were related to NAFLD clinical features such as body-mass-index and serum levels of triacylglycerol (TAG) and transaminases.
The functional role of *SLC19A1* was studied *in vitro* using shRNA-gene-knockdown technologies in THLE2-cells, which caused intracellular folate content 4-fold lower than in the wild type-cells. Furthermore, the lack of functional *SLC19A1* significantly altered the expression of critical genes leading to spontaneous lipid droplet accumulation within cells. Lipidomic analyses showed that the most abundant stocked lipids were TAG and cholesteryl-ester species (the main known components of intracellular lipid droplets) as well as polyunsaturated fatty acids.
We can conclude, for the first time, that genetic variants of *SLC19A1* are associated with NAFLD, and that the failure of this gene dysregulates critical genes for normal liver function, causing low intracellular folate levels and increased lipid accumulation.

PS06.27

Identification and characterisation of novel GLA mutations in Fabry disease

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Fabry disease (FD) is a rare, progressive, metabolic disorder of glycosphingolipid storage and is caused by mutations in the gene encoding the lysosomal hydrolase α-galactosidase A (GLA). In males, the disease usually leads to a classic spectrum of symptoms including cardiomyopathy, nephropathy, acroparaesthesia, cornea verticillata and gastrointestinal problems and, eventually, myocardial infarction, renal failure or stroke. Certain circumstances complicate the diagnosis of the disease. (1) The X-linked inheritance mode causes female heterozygotic mutation carriers to develop symptoms that are commonly milder and do not comprise the whole spectrum. (2) Mutations that do not completely abolish α-galactosidase A (AGAL) function usually manifest with milder disease.
In collaboration with Centogene AG/Rostock we frequently detect new variants where the clinical significance is unclear (VUS). We utilize cell culture-based over-expression of the mutant cDNAs in order to objectify the disease potential of the variants by their in vitro enzyme activity.
We introduce outline data for 25 patients harbouring novel GLA mutations (age of diagnosis, symptomatic spectrum) and oppose the data for in vitro enzyme activity and responsiveness to the pharmacological chaperone (PC) 1-Deoxygalactonojirimycine (DGJ), an alternate treatment for misfolding-prone AGAL variants.
We show increased prevalence of patients with GLA gene variants with an enzyme activity of >20%. Since these cases can lead to symptom onset later in life, a close clinical check-up is demanded. The in vitro enzyme activity system supports prediction of the pathologic progress of a given mutation and impacts decision-making on time and type of therapeutic intervention.

PM06.28

Clinical picture in Estonian patients with heteroplasmic m.3243A>G mutation in MT-TL1 gene

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The m.3243A>G mutation is known as the MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis and stroke -like episodes) mutation. However many clinical phenotypes associated with this mutation have been described including maternally inherited diabetes and deafness (MIDD).
The aim of the study was to describe clinical picture of Estonian patients with heteroplasmic m.3243A>G mutation referred to genetic councelling.
Results: the common m.3243A>G point mutation was detected in eight patients from three families. In all of them (8/8) progressive hearing loss was detected, followed by diabetes mellitus (7/8), migraine (6/8), psychiatric disorders (3/8), brain atrophy(3/8) and hypertrofic left cardiomyopathy (2/8). Most of our patients (7/8) had thin build and short stature.
Clinically MELAS syndrome was diagnosed in four patients from two families and MIDD in four patients from one family.
Conclusions: Our results confirm the clinical heterogeneity of the m.3243A>G mutation. Progressive hearing loss is a first and universally presented feature in our small cohort. Therefore m.3243A>G mutation should be considered in patients with (progressive) hearing loss. There are some evidence that m.3243A>G mutation causes similar clinical pictures within the family. But as our cohort is small this conclusion need further wider clinical investigation.

PS06.29

amamutdb.no: A relational database for *MAN2B1* allelic variants which compiles genotypes, clinical phenotypes, and biochemical and structural data of mutant MAN2B1 in α-mannosidosis.

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α-Mannosidosis (MIM# 248500) is an autosomal recessive lysosomal storage disorder caused by mutations in the *MAN2B1* gene, encoding the lysosomal α-mannosidase. The disorder is characterized by a range of clinical phenotypes of which the major manifestations are mental impairment, hearing impairment, skeletal changes and immunodeficiency. Here we report an α-mannosidosis mutation database, amamutdb.no, which has been constructed as a publicly accessible online resource for recording and analyzing *MAN2B1* variants. Our aim has been to offer structured and relational information on *MAN2B1* mutations and genotypes along with associated clinical phenotypes. Classifying missense mutations, as pathogenic or benign, is a challenge. Therefore, they have been given special attention as we have compiled all available data that relate to their biochemical, functional and structural properties. The α-mannosidosis mutation database is comprehensive and relational in the sense that information can be retrieved and compiled across datasets; hence, it will facilitate diagnostics and increase our understanding of the clinical and molecular aspects of α-mannosidosis. We believe that the amamutdb.no structure and architecture will be applicable for the development of databases for any monogenic disorder.

PM06.30

Gut microbiome composition is linked to metabolic improvements induced by dietary changes in Korean adoptees

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Introduction: A growing body of evidence suggests that healthy diet is one of the major factors associated with metabolic health and changes in gut microbiota. Here we conducted a low-salt, high-fiber diet intervention trial on Korean adoptees, to assess alterations in gut microbiome composition in response to changes in diet and the associations with metabolic improvement.
Materials and methods: We developed a modified version of traditional Korean diet (K-DASH), a high-fiber, low-fat, and low-salt diet with a sufficient supply of fruits and nuts. 19 Korean adoptees (mean age: 29.4 years, male: 44.4 %) whose diets are mainly western style were recruited. 16S ribosomal RNA genes were extracted from stool samples at both pre- and post-intervention trial. The V4 region was amplified by PCR and sequenced using Illumina MiSeq platform. QIIME v.1.9.0 was used to estimate taxa from the sequenced reads.
Results: The metabolic profiles, including weight, waist circumference, systolic blood pressure, high-density lipids, and triglyceride levels, showed an improvement, except for fast blood sugar level. The gut microbiome profiles at the phylum level, Firmicutes were decreased, while Bacteroidetes were increased in abundance. The operational taxonomic unit that showed the most significant increase in abundance belonged to the family Leuconostocaceae, which is known to produce acetate and lactate (FDR-corrected p = 0.0028). The change of triglyceride levels correlated with that of abundance of Actinobacteria (Pearson's correlation coefficient: 0.61, p=0.005).
Conclusions: Our findings suggest that metabolic improvements induced by a short-term changes of diet are mediated through their effects on the gut microbiome.

PS06.31

Role of ACBD3 protein in the mitochondrial energy metabolism

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A homozygous mutation c.460A>G in ACBD3 gene was found by whole-exome sequencing in patient with mitochondrial encephalomyopathy and combined deficiency of respiratory-chain complexes. ACBD3 encodes a 60 kDa acyl-coenzyme A binding domain containing 3 protein (also known as PAP7 or GCP60) localized in the Golgi apparatus, endoplasmic reticulum, and mitochondria. ACBD3 protein plays an important role in many cellular processes (lipid metabolism, membrane transport, neuronal division, embryogenesis, steroidogenesis, keeping the structure of the Golgi apparatus, apoptosis, iron homeostasis).
In mitochondria, ACBD3 protein is a part of the complex (140-200kDa) mediating cholesterol transport into the organelle. An integral part of this complex is VDAC protein (Porin) which forms freely permeable pores in the outer mitochondrial membrane and further associates with ANT protein (adenine nucleotide transporter) in the inner mitochondrial membrane. ANT is an ADP/ATP carrier that controls a pool of mitochondrial adenine nucleotides required for mtDNA maintenance.
The aim of the study is characterize the consequences of stably down-regulated expression of ACBD3 protein on the biogenesis and functions of OXPHOS complexes in HEK293 to elucidate its role in the pathogenesis of the patient´s phenotype. The first results show that deficit of ACBD3 protein leads to the decrease of amount and activity of complex IV and decrease of selected subunits of complex IV. Supported by research projects IGA NT/13114-4, GAČR 14-36804G, RVO-VFN64165/2012, and GAUK 1308214.

PM06.32

Identification of causal mutations in three Czech patients with cytochrome c oxidase deficiency and haematological disturbances

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Introduction: Anaemia represents a heterogeneous group of hematologic diseases, which can be induced by plenty of genetic and risk factors. Although the association of mitochondrial dysfunction and hematologic pathology is generally known, the underlying pathologic factors with regard to evolving anaemias are poorly characterized. In this study, we report on three patients, who manifested profound cytochrome c oxidase deficiency combined with anaemia.
Methods: Genetic causes of patients' defective OXPHOS system were found with the use of sequencing targeted to mitochondrial exome, application of SNP microarray chip and performance of protein studies in affected patient tissues.
Results: The presence of pathologic mutations was excluded in patients' mitochondrial DNA. A rarely occurring pathological 6-kbp homozygous deletion was identified in two unrelated patients affecting the *PUS1* gene, which remarkably leads to different disease-phenotypes in both patients. Two previously characterized deleterious missense sequence variations of *COX10* gene (p.Asn204Lys; p.Pro225Leu) were identified in the third patient, however, their combination has not been reported yet, which may imply the variant patient disease-phenotype.
Conclusions: Based on our results and current knowledge, we suggest the infantile deficiency of *PUS1* and *COX10* to be classified as the early fatal and slow progressive forms. To conclude, mitochondrial disorders manifest poor phenotype-genotype correlation even in the patient with the same causal mutations.
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PS06.33

Evaluation of results from a large NGS nuclear mitochondrial panel supports the use of NGS panels in cases of mitochondrial-like clinical features or non-specific presentations

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Next-generation sequencing (NGS) panels have expanded the number of genes that can be simultaneously investigated and are an attractive option because of their increased efficiency and reduced cost. Design of the 448 gene nuclear mitochondrial NGS panel included genes that are involved in various mitochondrial functions ranging from oxidative phosphorylation, transcription/ translation, mtDNA synthesis/biogenesis, and mitochondrial regulation. These genes also include those associated with conditions that

may mimic mitochondrial disease. We evaluated 43 Nuclear Mitome NGS cases to determine the most commonly reported and phenotypically informative genes. We reported Sanger confirmed variants in 372 of 448 genes. Overall about 65% of variants could be categorized into one of three mitochondrial disease associated subtypes: oxidative phosphorylation subunits, mitochondrial regulatory function, or conditions mimicking mitochondrial disease. About 43% of reported variants were in genes that cause similar clinical phenotypes, 20% were in genes that code for oxidative phosphorylation subunits, and 9.8% were related to regulatory function. The ten most reported genes (*RYR1, WFS1, POLG, SCN1A, MTFMT, TTBK2, ABCD1, MYH7, ATM,* and *ATXN7*) accounted for over 15% of all reported variants. Six of these ten genes are associated with conditions that may mimic mitochondrial disease. They may not be directly related to mitochondrial function but these patients' phenotypes prompted suspicion for mitochondrial disease demonstrating the importance of using NGS testing early in the diagnostic process for economic and efficiency purposes. Our results support the use of NGS panels to identify a potential diagnosis in cases of non-specific clinical features or mitochondrial disease-like symptoms.

PM06.34
Mitochondrial DNA mutation testing: how low can you go?

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The UK National External Quality Assessment Service (UK NEQAS) for Molecular Genetics has provided external quality assessment (EQA) for genetic testing laboratories since 1991. The Scheme challenged participants of the 2014 mitochondrial DNA (mtDNA) EQA run by distributing a validated sample with a very low level of mtDNA heteroplasmy (3% mutated mtDNA in circulating lymphocytes) of the m.3243A>G *MTTL1* mutation, resulting in a third of all participants reporting a critical genotyping error.
Thirty six percent of laboratories were either unable to identify the m.3243A>G mutation present at this low level or failed to state the sensitivity of their quantitative assay to determine mtDNA heteroplasmy in their report. Various testing methods were used for analysis and erroneous results were even reported by laboratories claiming to have test sensitivity as low as 1%.
The results of the EQA scheme highlighted that when reporting quantitative assessment of mtDNA mutation loads it is crucial that test sensitivity is included on reports to enable the reader to correctly interpret the result. Furthermore, if a diagnostic laboratory is using a test for the heteroplasmic m.3243A>G mutation which cannot reliably detect <10% mutant load then it is recommended that an alternative, more sensitive, testing strategy is employed or that an alternative, non-invasive DNA sample (e.g. urinary sediment) in which this mutation is known to segregate to higher levels, is routinely screened in patients referred for m.3243A>G testing.

PS06.35
Exome sequencing in a patient with suspected mitochondrial disease: the truth unveiled.

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As the recognition of mitochondrial diseases has increased, the spectrum of clinical manifestations has expanded to include organ systems such as gastrointestinal tract, kidney and liver. Here, we report our efforts to identify the pathogenetic cause for a patient, born to healthy consanguineous parents, who presented since the neonatal period a constellation of symptoms suggestive of a mitochondrial disorder: chronic diarrhea, failure to thrive, tubulopathy, hepatic cytolysis with a complex IV deficiency of the mitochondrial respiratory chain (MRC) in liver. Mutations in mitochondrial DNA and nuclear candidate genes have been excluded. Whole-Exome Sequencing (WES) of the patient identified a homozygous missense variant c.347G>A (p.Cys116Tyr) in the EPCAM gene, which is known to cause Congenital Tufting Enteropathy (CTE), a rare autosomal recessive diarrheal disorder. Additionally a homozygous missense mutation c.1870A>G (p.Thr624Ala) was identified in the SLC3A1 gene, which causes cystinuria, an autosomal inherited disorder characterized by impaired epithelial cell transport of cystine and dibasic amino acids in the proximal renal tubule and gastrointestinal tract. The two variants are novel, occur in highly conserved amino-acids, are predicted to be damaging and are present in the heterozygous state in the parents and the asymptomatic sister. This patient's complex phenotype reflects a complex genetic etiology in which no single gene explained the complete clinical presentation, but rather more than one mutant locus is involved. This study demonstrates the benefit of WES in providing the molecular diagnosis to patients with complex clinical presentations and in revealing a possible secondary MRC defect.

PM06.36
Unraveling the genetic cause of mitochondrial disorders by whole exome sequencing

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Mitochondrial disorders are often fatal multisystem disorders. Due to extreme clinical and genetic heterogeneity of these diseases, establishing a genetic diagnosis is challenging. Mitochondrial disorders can be caused by mitochondrial DNA (mtDNA) mutations, accounting for ~15% of cases, or by nuclear genetic defects. To date, mutations have been found in ~200 nuclear genes in mitochondrial patients but many disease genes still have to be discovered.
Development of whole exome sequencing (WES) has revolutionized our approach to uncover disease-causing mutations in known and novel mitochondrial disease genes. We performed WES analysis on ~50 (consanguinous or non-consanguinous) patients with suspected mitochondrial disease and no mtDNA or POLG1 mutations, filtering the exome data based on the presumed genetic model (mostly autosomal recessive). So far, in ~35% of these patients a clear (~25%) or probable (~10%) genetic cause has been identified; most (~2/3) of these are mutations in known mitochondrial genes while ~1/3 are not previously linked to mitochondrial disease.
Therefore, WES has now been implemented in our laboratory as a diagnostic test for patients with suspected mitochondrial disease, starting with a mitochondrial gene panel of ~450 genes for initial filtering. In case the causal genetic defect is not found, filtering the complete exome data ('Open Exome') based on the presumed genetic model can be performed as a second step. For most novel candidate genes and mutations, however, functional testing is required to prove pathogenicity or, alternatively, identification of similar gene mutations in patients with comparable phenotypes, demonstrating the value of data sharing.

PS06.37
Particularities of mitochondrial DNA connected with manifestation of muscle tissue hypoxia in congenital myopathies.

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Introduction
The confirmation or exclusion of mitochondrial diseases remains a challenge in clinical practice, especially for pediatric cases, which show enormous variation in clinical presentations. Demonstrative example are infant myopathies. Different manifestations of congenital myopathy may mask mtDNA alterations. Oftimes mitochondrial disorders are not suspected and manifest unexpectedly. In this regard, molecular mtDNA testing is getting increasingly important as a stage of diagnostics.
Materials and methods
We researched unexplained infant myopathy cases with manifestation of muscle hypoxia. First, we analyzed mitochondrial distribution in the tissue. Then, we explored distribution of Hypoxia Inducible Factor 1 alpha (HIF1a) to evaluate hypoxia. For this stage, we used polyclonal rabbit antibodies to HIF1a and secondary antibodies AlexaFluor555 goat anti-rabbit IgG. Thereafter we made complete mtDNA sequence with Sanger method and capillary electrophoresis.
Results
Histological research of muscle tissue revealed ragged red fibers. More interestingly, we found loci with both HIF1a high concentration sites and increased mitochondrial clusters. In the light of these findings, we analyzed complete mtDNA sequence in order to find pathological mutations. We found no confirmed disease-associated mutations. Yet in the full mtDNA sequence we found several novel variants in each patient: A191T, T199C, T961C, T3197C, A4769G, T7080C, G8251A, A8860G, T11770C, A15218G, A15326G, A16080G, T16209C, C16294T, C16256T, T16519C (no connection with haplogroup).

Conclusions
We expect novel individual mtDNA variants to be connected with mitochondrial disorders and local manifestations of muscle tissue hypoxia. We suppose it is crucial not only to check mtDNA "hot points" but also to make complete mtDNA sequencing to evaluate novel genetic variants in cases of unexplained infant myopathies.

PM06.38
Association between seminal protein oxidation and mitochondrial membrane potential of human spermatozoa from infertile men

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Introduction: Sperm mitochondria are one of the major sources and targets of reactive oxygen species (ROS). Furthermore, mitochondrial dysfunction is reported to be an early indicator of sperm damage. The aim of this study was to evaluate the influence of oxidative stress, as assessed by seminal oxidation protein level, on mitochondrial membrane potential (MMP) of sperm.
Materials and Methods: Study population included 25 male partners of infertile couples. Were excluded men with known male factor of infertility and/or low ejaculate volume (<1.5 mL) and/or low sperm count (< 15 Million/mL), evaluated according to WHO 2010 recommendations. MMP was determined by flow cytometry using 3,3'-dihexyloxacarbocyanine iodide (DiOC6) as probe. Seminal plasma level of advanced oxidation protein products (AOPP), a marker of oxidative stress, was assessed by spectrophotometry. The relationship between semen parameters, sperm MMP and AOPP levels were analyzed (Spearman's Rank correlation test).
Results: Nearly one-third of the participants (n=8) had normal semen parameters. Among the remaining participants, 70 % had a single parameter below WHO reference values. Sperm progressive motility was significantly correlated with MMP (r=+0.44; p=0.02). We found a negative correlation between AOPP seminal level and sperm concentration (r= -0.40; p=0.04). There was no association between AOPP level and MMP (p=0.9).
Conclusion: In accordance with previous studies, sperm MMP was a sensitive indicator of functional integrity of the spermatozoa. The lack of association between MMP and AOPP level could be related to the relatively good semen quality of the participants in this study. Indeed, semen vulnerability to oxidative damage is strongly linked to semen quality and its antioxidant capacity.

PS06.39
Molecular diagnosis of monogenic diabetes by targeted next-generation sequencing in Slovak MODY patients

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Maturity Onset Diabetes of the Young (MODY) is genetically heterogeneous disorder. Generally, only few genes with most prevalent mutations are sequenced according to patient phenotype. In this study, we used targeted NGS sequencing of a panel of MODY genes in patients, where no mutation had been found in one or two most probable genes by Sanger sequencing.
Patients and Methods: We designed a custom targeted sequencing panel focusing on 13 known MODY genes (HNF4A, GCK, HNF1A, PDX1, HNF1B, NEUROD1, KLF11, CEL, PAX4, INS, BLK, ABCC8, KCNJ11). The target regions (exons with 50bp padding, UTRs and promoters) were sequenced using PGM IonTorrent platform. Missing and low coverage fragments, as well as candidate variants, were sequenced by Sanger. We have analyzed 21 patients with MODY phenotype negative for most probable genes.
Results: Designed panel covered 93.7% of target sequences and, out of these, 98.1% were covered more than 20x. Analysis of data revealed 3 known mutations: in GCK, ABCC8 and HNF1B genes, and 2 novel likely pathogenic mutations in ABCC8 and HNF4A genes. We have also found additional 3 variants in HNF1B, PAX4 and BLK genes with unknown significance. Co-segregation analysis with diabetes in the families did not support their pathogenicity.
Conclusions: Our first results from targeted NGS data showed an effective usage of this method in the routine DNA diagnostics of MODY in Slovakia. We have solved 5 out of 21 cases (25 %) of patients, who would not be diagnosed by classical approach due to their atypical phenotype.
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PM06.40
A new MRM-MS assay for the diagnosis of mucopolycaccharidosis type IVA (Morquio A disease) in dry blood spots

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Mucopolysaccharidosis IVA (MPS IVA; Morquio A disease) is an autosomal recessive disease caused and characterized by an impaired activity of galactosamine-(N-acetyl)-6-sulfate-sulfatase (GALNS), resulting in keratan sulfate and chondroitin-6-sulfate accumulation in tissues and secondary organ damage. Enzyme replacement therapy, currently in clinical trials, renders the identification of MPS IVa patients in a rapid and facile manner of outmost importance. We propose a newly developed assay for the stabile and reproducible detection of GALNS deficiency in dry blood spots (DBS). Blood samples were taken from 57 healthy individuals and 18 randomly selected MPS IVA patients. The patients were presenting the MPS IV A phenotype and the genetic test revealed GALNS gene mutations, ten of which previously not described in literature. The material extracted from DBS was incubated with a 4-methylumbelliferyl-β-D-galactopyranoside-6-sulfate as a specific synthetic substrate. Final enzymatic product, 4-methylumbelliferone, obtained after adding exogenous beta-galactosidase, was quantified by LC/MRM-MS (liquid-chromatography/multiple-reaction-monitoring mass-spectrometry). 4-propyl-5-hydroxy-7-methyl-2h-chromen-2-one was used as internal standard, a compound with a very similar molecular structure and fragmentation pattern in negative ion mode as 4-methylumbelliferone (the enzymatic product in the present assay). The cut-off was established at 4.8 µmol/L/h. The enzymatic assay yielded a positive and negative predictive value of 1.0 for genetically confirmed MPS IVa patients (with a GALNS activity of 0.35 ± 0.21 µmol/L/h) compared with controls (normal GALNS activity 23.1 ± 5.3 µmol/L /h). Results show a pathologically reduced GALNS activity in all MPS IVa patients, while the control enzymes show always normal activity.

PS06.41
Mutation analysis of the MRAP2 gene in Prader Willi like patients and obese children and adolescents.

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Background: In general, there is increasing recognition that accessory proteins can modulate G protein-coupled receptor (GPCR) trafficking, ligand binding and signaling to the cell surface. *In vitro* studies showed that melanocortin receptor 2 accessory protein 2 (MRAP2) can interact with the melanocortin 3 (MC3R) and 4 receptor (MC4R), two GPCRs which play an important role in the leptin-melanocortin signaling. *MRAP2* is located on chromosome 6q14.2, a region which is associated with a Prader Willi like (PWL) phenotype, a syndromic form of obesity. In addition, Asai *et al.* identified four rare heterozygous variants in four unrelated, nonsyndromic, severely obese individuals. Therefore we decided to perform mutation analysis in both a PWL and obese cohort.
Method: We screened 122 PWL patients and 404 obese children and adolescents for mutations in *MRAP2* using high-resolution melting curve analysis. Sanger sequencing was performed for samples with melting patterns deviating from wild-type.
Results and conclusions: Mutation analysis of all coding exons and intron-exon boundaries of *MRAP2* resulted in the identification of one rare non-synonymous heterozygous variant A40S (c.118G>T) in one PWL patient. Screening of the 404 obese individuals didn't lead to any variants in the gene. *In silico* analysis showed a probably damaging effect of the A40S variant on the protein structure of MRAP2. Further functional analysis is necessary to investigate the influence of the variant on the MRAP2 function. This would indicate a possible role of *MRAP2* in the pathogenesis of Prader Willi like phenotype, albeit in a limited number of patients.

PM06.42
'Early treatment with Elaprase: Can it be effective in neuropathic form of MPS II?'

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Introduction: Enzyme replacement therapy (Elaprase) has been used for treatment of patients with mucopolysaccharidosis type II (MPS II) for the last eight years. During this time only few early-diagnosed infants have been

treated. Therefore, data on treatment effectiveness in this age group is very limited.
Case presentation: We present unexpected therapeutic findings in one of two affected siblings. In the older patient first symptoms of MPS II were noticed at the age of three years and diagnosis was established two years later. At the age of seven years, typical dysmorphism, restriction of joint movements, heart involvement, hepatomegaly and severe intellectual delay could be observed. In the younger patient diagnosis of MPS II was made in the first month of life. Treatment with Elaprase was introduced in the third month of life. At the age of seven years the patient remains clinically symptom-free (including normal intellectual development) despite presence of typical biochemical abnormalities. In both patients activity of iduronate 2-sulfatase in leukocytes was below norm and a missense mutation of the iduronate 2-sulfatase gene (p.Tyr523Cys) was detected.
Conclusion: Although hampering of some symptoms could be explained with beneficial effects of Elaprase, normal intellectual development in the younger patient is unexpected. One possible explanation could be decreased disease penetration. However, similar extent of biochemical abnormalities in both patients suggests similar disease severity. Therefore, in our opinion possibility of significant attenuation of disease course should be considered as a result of early introduction of enzyme replacement therapy.

PS06.43

Severe TK2 enzyme activity deficiency in patients with mild forms of myopathy.

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Thymidine kinase 2 (TK2) is a mitochondrial enzyme participating in the salvage of deoxyribonucleotides needed as substrates for mitochondrial DNA (mtDNA) replication. TK2 mutations were typically associated with a severe myopathic form of mtDNA depletion syndrome that manifests during infancy and leads to the early death of most patients. Recently, several patients (n=7) have been diagnosed with a late-onset and milder myopathy with multiple mtDNA deletions. Here we describe seven patients diagnosed in their adulthood (16-55 years old), with different forms of mitochondrial myopathy associated with multiple mtDNA deletions and no depletion in skeletal muscle, in contrast to what is observed in typical infantile patients. Phenotypic presentation varied from mild myopathic signatures, such as ptosis and myalgia, to progressive muscle weakness and respiratory dysfunction. After genetic analysis, previously reported pathogenic mutations in TK2 were identified in all patients (p.K202del and p.T108M that seem particularly frequent in the Spanish population, and p.R192K). Fibroblasts were obtained from two of these patients and TK2 activity was measured showing a drastic reduction that resembled that found in typical paediatric patients (3% and 6% residual activity as compared with age-matched healthy controls). This observation suggests that redundant or complementary biochemical mechanisms can bypass the biochemical defect in some individuals, preventing mtDNA depletion, and ameliorating the course of the disease. Therefore, TK2 mutations should be investigated in patients with myopathy associated with either mtDNA depletion or multiple deletions, independently of their age at onset or clinical severity.
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PM06.44

Homozygous mutations in MFN2 are a novel cause of multiple symmetric lipomatosis in patients without a MERFF mutation

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Multiple symmetric lipomatosis (MSL) is a mitochondrial disorder with impaired brown fat metabolism that has been associated with MERFF mutations in some, but not all patients. We studied a sibling pair and an unrelated individual who presented with multiple symmetric lipomatosis (MSL) and neuropathy to determine the genetic etiology of this disorder in patients who did not carry the MSL-associated MERFF mutation. The siblings presented with striking cervico-thoracic lipomatosis in their 40's, with tongue hypertrophy and impaired swallowing, diabetes, and a peripheral neuropathy. Whole exome sequencing was performed on the siblings and a rare, shared homozygous mutation in *MFN2* (c.2119C>T: p.R707W) was identified. The mutation was not present in their healthy siblings. In silico programs predict it to be pathogenic and heterozygous carriers of the MFN2 p.R707W substitution are known to have Charcot-Marie-Tooth disease (CMT). A third, unrelated, patient with multiple symmetrical lipomatosis and neuropathy also harbored the same homozygous mutation and had been previously diagnosed with CMT. Functional studies in patient fibroblasts demonstrate that the p.R707W substitution impairs homotypic (MFN2-MFN2) protein interactions required for normal activity, and renders mitochondria prone to perinuclear aggregation. These findings show that homozygous mutations at p.R707W in *MFN2* are a novel cause of multiple symmetrical lipomatosis.

PS06.45

A homozygous p.Trp22Arg *NDUFB3* mutation is a recurrent cause of mitochondrial complex I deficiency associated with Irish ancestry, a characteristic facial appearance and good prognosis

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Isolated complex I deficiency is the most common paediatric mitochondrial disease presentation and the prognosis is often poor with high mortality. Complex I comprises 44 structural subunits with at least 10 ancillary proteins involved in its assembly; mutations in at least 25 of these have been reported as causal. There are limited genotype-phenotype correlations to guide clinicians to the correct genetic diagnosis, although next-generation sequencing strategies including exome sequencing have proven extremely useful in a research setting. In a diagnostic setting, it is crucial to provide a genetic diagnosis in a rapid and cost-effective manner and we apply a validated, targeted next-generation sequencing strategy to sequence the genes encoding all structural subunits and ancillary proteins in clinically- and biochemically-characterised paediatric patients. This strategy has uncovered a clinically-distinctive cohort of children of small stature, with persistent growth retardation and mild dysmorphic facial features (n=7 patients from 6 families) who are all homozygous for a previously reported c.64T>C, p.Trp22Arg *NDUFB3* mutation. Two further children from a seventh family were referred after exome sequencing identified the same homozygous p.Trp22Arg *NDUFB3* mutation; these children presented with primordial short stature without obvious metabolic dysfunction. The p.Trp22Arg mutation has previously been associated with severe metabolic presentations and poor prognosis. Our cohort highlights that the long-term prognosis related to this *NDUFB3* mutation can be very good, and recognition of the distinctive facial features should suggest screening for this specific mutation to provide a genetic diagnosis, circumventing the requirement of muscle biopsy to direct molecular genetic investigations.

PM06.46

Newborn screening for fatty acid oxidation disorders: effects on population frequency and clinical outcome in the Czech Republic

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Introduction: Newborn screening (NBS) program in the Czech Republic was

expanded from three to 13 disorders in October 2009 and tests also for the medium chain acyl-CoA dehydrogenase (MCADD) and long chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHADD). In this study we examined the effect of five years of NBS on detection rate and clinical outcome compared to the pre-NBS period.
Patients and Methods: In the pre-NBS cohort 21 MCADD and 12 LCHADD patients were ascertained on clinical basis and followed for 236 and 73 patient-years, respectively. In the NBS cohort 29 MCADD and 10 LCHADD patients were detected among 661,000 newborns with a follow-up of 74 and 26 patient-years, respectively. A severity scoring index (SSI) was developed to assess the clinical outcome. Statistical analyses were carried out using statistical environment R.
Results: The NBS increased significantly the frequency of ascertained patients with MCADD from 1:211,300 to 1:22,800 and with LCHADD from 1:141,300 to 1:66,100. A total of 12 and 8 variants in the ACADM and HADHA genes were detected and a different spectrum of mutations was observed between the NBS and clinically ascertained cohorts. The age-adjusted clinical SSI in the pre-NBS cohort of MCADD patients (median 0.8 versus 0.0, p=0.009, Wilcoxon test) and LCHADD patients (median 3.5 versus 0.4, p=0.011, Wilcoxon test) were significantly higher compared to the NBS cohort.
Conclusions: Five years of newborn screening for MCADD and LCHADD in the Czech Republic significantly increased detection rate and improved clinical outcome in patients.
Study was supported by project MH-CZ-DRO-VFN64165.

PS06.47
NGS BASED IDENTIFICATION OF HYPERCHOLESTEROLEMIA RELATED MUTATIONS IN SUBJECTS WITH INCREASED LDL-C LEVELS

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Familial hypercholesterolemia (FH) is one of the most common monogenic disorders mostly inherited as autosomal dominant trait and when untreated results in early coronary heart disease. Identification and early treatment of affected individuals remains a challenge worldwide. Majority of FH cases are caused by mutations in four genes (APOB, LDLR, PCSK9 and LDLRAP1). The spectrum of disease causing mutations is very diverse and mutation panels commonly used in diagnostics cover only a minority of disease causing genetic variants. In FH patients not expressing the physical symptoms and with unknown family history DNA based tests may provide the route to FH diagnosis. Here, we evaluate the use of targeted next generation sequencing (NGS) as a potential method to identify cases of FH in the cohort of coronary artery disease (CAD) patients and individuals with abnormal low density lipoprotein cholesterol (LDL-C) levels.
We used targeted amplification of LDLR, APOB, PCSK9 and LDLRAP1 coding regions followed by NGS in 42 CAD patients (LDL-C 4.1-7.2 mmol/L) and 50 individuals from population based cohort (LDL-C 5.1-9.7 mmol/L). In total 22 synonymous, 31 non-synonymous variants, eight variants in close proximity (10 bp) of intron-exon boundaries and 50 other variants were found. We identified 4 pathogenic mutations (Arg3527Gln in APOB gene, Gly20Arg, Arg350X and IVS11 -10 (G>A) in LDLR gene) in seven patients (7.6 %). In addition 3 possible pathogenic variants were also found in four patients.
This work was supported by European Regional Development Fund „Research and development of novel diagnostic methods for genetic and immunologic testing" no 2013/0042/2DP/2.1.1.1.0/13/APIA/VIAA/002 and State Research Project "BIOMEDICINE".

PM06.48
Development of an improved diagnostic service for Niemann Pick type C

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Niemann-Pick disease type C (NPC) is a rare autosomal recessive lysosomal storage disorder, resulting from abnormal cholesterol trafficking. NPC has a heterogeneous clinical presentation, but is typically characterised by a range of progressive neurological problems.
Traditionally, a biochemical laboratory diagnosis is made by demonstrating impaired cholesterol trafficking via filipin stained fibroblast cultures, requiring an invasive skin biopsy. A more sensitive and specific LC-MS/MS method for quantifying cholesterol oxidation products, shown to be increased in the plasma of NPC patients is now available in Manchester as a new first line test, allowing less invasive, rapid screening of this previously underdiagnosed disease.
Currently, molecular analysis of NPC following abnormal biochemical screening results, involves direct Sanger sequencing of all 30 exons of the *NPC1* and *NPC2* genes combined. However, a more robust efficient approach was required to meet the potential increase in demand for NPC genetic testing.
We have developed a more comprehensive sensitive mutation screening service for both *NPC1* and *NPC2* using a combination of MLPA and Next Generation Sequencing (NGS). The latter is based on enrichment by long-range PCR, amplicon normalisation, equimolar pooling and dilution, fragmentation and tagging using Nextera XT followed by MiSeq NGS, with a minimum coverage of 100x reads. Analysis using a custom bioinformatics pipeline allows a range of point variants and small insertion/deletion mutations to be accurately identified.
In addition, the cholesterol oxidation levels from a number of patients were compared to their genotypes and good concordance between the two tests was revealed.

PS06.49
Lyso-Sphingomyelin-509 as sensitive and specific biomarker for Niemann-Pick Type C Disease

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Introduction: Biomarkers are a relevant factor in diagnosing and monitoring many different diseases. The ideal biomarker detects the presence, extent and outcome of a specific disease and is reliably quantifiable in a clinically easily accessible sample. For Niemann-Pick Type C (NP-C), an autosomal-recessive disease caused by mutations in either the NPC1 gene (95% of all patients) or NPC2 gene (5% of all patients) biomarkers have been established in the past, which facilitate either early diagnosis (cholestane-3β,5α,6β-triol; Jiang et al. 2011) or long-term follow-up (Lysotracker Assay measuring relative acidic compartment volume; TeVruchte et al., 2014). However, early diagnosis and monitoring of treatment in patients is still difficult and the existing markers are not really reflecting the disease burden.
Material and Methods: Based on a new LC/MRM-MS (liquid-chromatography/multiple-reaction-monitoring mass-spectrometry) assay we developed and validated a novel biomarker, lyso-Sphingomyelin-509. Primary detection of the biomarker was performed in 10 NP-C patients and 10 controls. Further validation of the biomarker was performed in 242 subjects including NP-C patients, NP-C carriers, healthy controls and patients with other LSDs.
Results: In total, 242 subjects were investigated, including 110 NP-C patients (lyso-Sphingomyelin-509: 6.7ng/ml (IQR: 3.4-10.3ng/ml)), 63 NP-C carriers and 43 healthy controls. With a sensitivity of 100% and specificity of 91% a cut-off of 1.4ng/ml was established. Neither NP-C carriers nor healthy controls displayed elevated measurements of lyso-Sphingomyelin-509.
Conclusion: Lyso-Sphingomyelin-509 has been established as a sensitive and specific biomarker for NP-C. Tracking the disease progression and response to treatment by this surrogate biomarker is currently under investigation.

PM06.50
Recurrence of Niemann-Pick Type C in extended families with no consanguinity

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Niemann-Pick Disease Type C(NPC) is a rare neurodegenerative lysosomal storage disorder with an incidence of about 1:120,00. Inheritance is autosomal recessive. There are less than 90 cases in the United Kingdom. This abstract presents 3 families with no consanguinity. In 2 families first cousins had affected children and in a third 2 sisters had an affected child. Given the published low incidence of this disorder possible explanations for these events will be explored. Given that families with NPC are supported by a very good charity (NP UK) and also that networks amongst affected families, particularly of late with the ever increasing use of social media, are very strong, knowledge of these recurrences in extended families are known about which impacts markedly when offering genetic counselling.

PS06.51

The next-generation of the Niemann-Pick Type C Disease Gene Variation Database (NPCdb)

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Niemann-Pick Type C Disease (NP-C) is a rare, autosomal-recessive lysosomal storage disease. Two causative genes, NPC1 and NPC2, have been identified with mutations in NPC1 contributing to the large majority of cases.
In 2007 we established a locus-specific gene variation database that listed variants and associated haplotypes in NPC1 and NPC2 genes and related sequence to basic clinical information. Since then our knowledge has grown and next-generation sequencing studies of larger patient and control cohorts have provided us with further insights into the genetic architecture of NPC1 and NPC2, enabling a much better understanding of which genetic variation within these genes is truly disease-relevant.
Using a variety of sources, we have now assembled over 1400 exonic and intronic variants at the NPC1 and around 50 variants at the NPC2 locus and provide information on their functional consequences. Additionally, our resource provides information on allele frequencies, in silico predictions of the functional effects of a variant and allele-numbers in controls and reported patients. As a result it assists in evaluating the significance of a variant and thus aims to support molecular diagnostician in their challenge of making the correct diagnosis.
In recent years, efforts have been made to establish a clinical registry for Niemann-Pick diseases called "The International Niemann Pick Disease Registry". As we are collaborators in this project we aim to intertwine both registries, thus securing an ongoing input of information on the number and types of variants found in actual NP-C patients.
We are planning to launch this new resource for Niemann-Pick Disease type C in spring 2015.

PM06.52

Positive correlation between orphan receptor RORα and ABCA1, ABCG1 in visceral adipose tissue

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Obesity is associated with impaired lipid metabolism and cholesterol accumulation in the intra-abdominal adipose tissue. ATP-binding cassette transporters ABCA1 and ABCG1 play a major role in cholesterol efflux suggesting their role in elimination of excess cholesterol and maintaining cholesterol homeostasis in the adipocyte. RAR-related orphan receptor RORα have been implicated as one of the regulators of the lipid metabolism and may play a significant role in the regulation of ABCA1 and ABCG1 expression. However, the effect of RORα on ABCA1 and ABCG1 protein content in visceral fat during obesity is unknown.
The aim of this study is to investigate correlation between orphan receptor RORα and transporters ABCA1, ABCG1 in visceral adipose tissue with obesity.
Visceral fat was received from gastrocolic omentum during laparoscopic cholecystectomy from 37 individuals. Protein levels of ABCA1, ABCG1 and RORα were measured by western-blot.
All individuals were divided in 3 subgroups: morbidly obese (BMI≥31; N=8), overweight (25≤BMI<31; N=15) and normal weight (BMI<25; N=14). ABCA1 and ABCG1 protein levels were significantly higher in individuals with overweight when compared with normal weight ($p<0.01$; $p<0.05$, respectively). A positive correlation between RORα and ABCA1(-r=0.48; $p<0.05$) as well as ABCG1(-r=0.43; $p<0.05$) levels in visceral fat was shown. These data suppose that RORα may control ABCA1 and ABCG1 protein levels in visceral fat tissue and thus influence on the obesity development.
The research was supported by RFFR 14-04-31690 grant.

PS06.53

High prevalence of monogenic obesity in super obese individuals undergoing bariatric surgery

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Introduction: Some forms of obesity are monogenic. We investigated the prevalence of monogenic obesity in super obese adults, and examined their weight loss trajectories after bariatric surgery.
Methods: A cohort of patients (n=282) with a body mass index (BMI) of >50 kg/m^2 were screened for MC4R deficiency. 48 patients (BMI range: 50.2-103.8 kg/m^2) were selected for further whole exome sequencing (WES) to screen for other pathogenic mutations in known obesity genes. Copy number variation analysis was carried out by read depth analysis, and Sanger sequencing or real-time PCR was used to confirm findings.
Results: Screening identified one patient carrying a rare *MC4R* mutation (E42K) predicted to be pathogenic. Five out of 48 patients (10.4%) investigated by WES carried a likely monogenic cause of their obesity: 16p11.2 deletion; a deletion within *NTRK2*; a nonsense mutation in *IGSF1*; and missense mutations in *SH2B1* and *NTRK2*. In addition, in a further six patients, novel or rare (population frequency <0.005) missense mutations, reported as pathogenic by multiple prediction tools, were found in *LEPR* (compound heterozygote), *NTRK2*, *SH2B1* or *MAGEL2* (three). Weight loss seen in the mutation carriers was not different one year after bariatric surgery from patients with a similar starting BMI.
Conclusion: *MC4R* screening and WES data revealed a markedly higher diagnostic yield (up to 22.9%) than previously reported in any obese cohort. Monogenic obesity is underestimated in the super obese.
This work was funded by a grant from Diabetes UK to AB and RW, and Biomedical Research Centre awards to AB, RW, MvH and ClR.

PM06.54

Heterozygous SDHA mutation resulting in complex II deficiency with ocular, cardiac and neurologic involvement

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Mitochondrial respiratory complex II (succinate dehydrogenase, SDH, CII) deficiency is rare, accounting for ~2% of all respiratory chain deficiencies. Of the four nuclear encoded proteins composing CII (SDHA, SDHB, SDHC, SDHD) and its assembly factors (SDHAF1 and SDHAF2) mainly recessively inherited mutations cause mitochondrial disease phenotypes.
Here we report clinical, biochemical and molecular investigations of three patients harbouring a heterozygous mutation in the flavoprotein subunit SDHA previously associated with CII deficiency. The index patient presented with optic atrophy, ocular movement disorder, progressive polyneuropathy, psychiatric involvement and cardiomyopathy. His daughter deceased at the age of 7 months due to cardiac insufficiency. His 30-year-old son presents with optic atrophy and cardiomyopathy. Spectrophotometric and oxygen consumption measurements in skin fibroblasts revealed isolated CII deficiency in all patients. Sequencing of all complex II associated genes revealed a previously described heterozygous mutation (c.1351C>T; p.R451C) in SDHA in a highly conserved Flavin-binding domain.
Only few SDH gene defects are reported as an underlying cause of mitochondrial disease. However, SDH genes as tumor suppressor genes are frequently associated with hereditary cancer syndromes, including paraganglioma and pheochromocytoma and should be monitored also in patients presenting with mitochondrial disease. Contrasting to previously reported cases, our patients present with cardiomyopathy. The variability in disease-onset and phenotypic presentation of this mutation, therefore, suggest modifying factors influencing disease severity. Our report gives further evidence for an autosomal-dominant inherited mitochondrial disease caused by a heterozygous SDHA mutation.

PS06.55

Functional and structural analysis of novel missense mutations of phenylalanine hydroxylase

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Phenylketonuria (PKU) and hyperphenylalaninemia are the most frequent disorders of amino acid metabolism. This group of disorders is primarily caused by mutations in human phenylalanine hydroxylase gene (PAH), leading to decreased or deficient enzyme activity. Phenylalanine hydroxylase (PAH) catalyzes the essential conversion of phenylalanine to tyrosine in the presence of molecular oxygen, iron and its natural cofactor tetrahydrobi-

opterin (BH4). In vitro analysis of PAH enzymes enables to determine the catalytic function of different mutated forms of PAH, thus to confirm that the amino acid change is the disease causing mutation. This is important in recent progress of PKU treatment and in the correlation between PAH residual activity and tetrahydrobiopterin therapy. The most worldwide frequent mutations of the PAH gene have been studied in vitro and in silico in the last two decades. In our recent study, three novel missense mutations were identified in the Slovak population (p.F233I, p.R270I and p.F331S). We cloned these variants in the pMAL-MBP fusion vector and expressed in E. coli XL1 blue expression system. Enzymes were isolated using affinity chromatography followed by size-exclusion chromatography. Wild type PAH and each mutated form were assayed and their activity was measured by quantification of produced tyrosine using tandem mass spectrometry. The residual activity of each mutation was assessed as percentual activity of the wild type enzyme. To clarify and extend the results, we changed experimental conditions by co-overexpressing each mutation with pGroESL plasmid (carrying chaperone genes GroEL and GroES). In addition, we performed structural analysis of all mutations using molecular dynamics simulations. This project was sponsored by grant APVV-0240-12.

PM06.56

Molecular heterogeneity of PAH gene in PKU patients from Republic of Moldova

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Phenylketonuria (PKU) is an inherited metabolic disorder, autosomal recessive (OMIM 261600), caused by deficiency of phenylalanine hydroxylase (PAH, EC 1.14.16.1) that leads to severe mental retardation. The aim of our research was to appreciate prevalence of PKU in Moldavian population and of PAH gene mutations identified in PKU patients.
Materials and Methods: Research was performed on DNA collected from 99 Moldavian PKU patients accumulated during 25 years. PKU detection by neonatal screening was based on the fluorimetric method. Identification of 10 PAH gene mutations (R408W, P281L, R158Q, R261Q, R252W, IVS12+1G>A, L48S, R261X, G272X, IVS10-11G>A) was performed by PCR/RFLP.
The results: According to neonatal screening data the frequency of PKU in Republic of Moldova is 1:7325 newborns, the average screening rate being 75.2%. In PKU patients were identified following genotypes: R408W/R408W (27.6%), R408W/P281L (7.1%), R408W/R261Q (3.5%), R408W/L48S (3.5%), R408W/R158Q (2.3%) etc. The most frequent PAH gene mutation in Moldavian PKU patients is R408W (50,6%) followed by P281L (5.5%), L48S (4.9%), R261Q (3.1%), R158Q (3.1%), R252W (3.1%), IVS12+1G>A (2.4%), IVS10-11G>A (2.4%), R261X (1.2%) and G272X (0.6%). The mutation detection rate in PKU patients constituted 78.7% of the cases. L48S, R261X, G272X, IVS10-11G>A mutations are recently investigated in Moldavian PKU patients due to their high prevalence in neighboring populations as Serbia, Romania, Turkey etc.
Conclusion: The obtained data revealed a high degree of heterogeneity of Moldavian PKU patients in the PAH locus that could be the result of population heterogeneity open new possibilities for prenatal diagnostics in risk families.

PS06.57

Intractable seizures and tendency for cerebral and portal vein thrombosis in a family with inherited Glycosylphosphatidyl inositol deficiency due to a mutation in *PIGM*

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Defects of the glycosylphosphatidylinositol (GPI) biosynthesis pathway constitute an emerging group of inherited disorders of heterogeneous phenotype, which are a subgroup of congenital disorders of glycosylation. A mutation in the promoter of *PIGM* has been identified as the cause of portal vein thrombosis and persistent absence seizures in three patients known to date. In one patient, targeted treatment with Sodium-Phenylbutirate has been described.
We report of two siblings, born to consanguineous parents of Arab-Muslim descent, who presented with macrocephaly, absence seizures, and gastrointestinal bleedings due to portal vein thrombosis. An extensive diagnostic evaluation was futile, including Whole Exome Sequencing (WES) which failed to establish a diagnosis. However, focused sequencing of the *PIGM* gene including its promotor region found the patients to be homozygous for the -270C>G mutation previously described. One of the siblings died awating liver transplantation, while the other is treated with Sodium-Phenylbutirate with improvement in her seizure frequency and severity. Finally, during a new pregnancy, prenatal diagnosis was pursued via amniocentesis, and unfortunately had found the fetus as homozygous for the same mutation. A third affected sibling was born, and at 7 months of age had already shown portal vein thrombosis, as well as evidence of cerebral infarct, and Sodium-Phenylbutirate treatment was initiated.
In conclusion, we expand the current knowledge on *PIGM*-associated GPI deficiency, with further clinical, pathological and molecular delineation of this unique entity, including the first prenatal diagnosis, and additional insights into the targeted-treatment with Sodium-Phenylbutirate. This case also underscores one of the pitfalls of WES.

PM06.58

POLG mutations in late-onset ataxia of unknown origin

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Mutations in the mitochondrial DNA polymerase gamma (*POLG*) gene cause a highly heterogenic disease spectrum including inherited ataxias. The reports about their frequency in unclassified forms of ataxia yield equivocal results, thus leading to uncertainties about the role of *POLG* in these conditions. The most common pathogenic *POLG* variants associated with ataxic phenotype are c.1399G>A (p.A467T), c.2243G>C (p.W748S) and c.2542G>A (p.G848S).
We assessed the frequency of selected *POLG* mutations in a cohort of 427 individuals (211 females and 216 males) with clinical symptoms of late-onset spinocerebellar ataxia (SCA), in whom the most frequent dynamic mutations causing autosomal dominant SCA (SCA1, 2, 3, 6) were excluded. DNA samples were obtained from leukocytes of peripheral blood. Genotyping of the samples was performed by real-time PCR technique using specific TaqMan allele discrimination assays.
Two heterozygous carriers and one p.W748S homozygote were identified in the studied group. The 52-year old patient presented with progressive cerebellar syndrome, sensory-motor polyneuropathy and ophtalmoplegia with atrophy of cerebellum, and less pronounced atrophy of the brain stem in neuroimaging examination. The age of onset was 30. Positive family history was reported: his brother demonstrated similar clinical signs and his sister died at 33 years due to the epileptic seizures.
Our findings are in line with the literature data and confirm that *POLG* mutations are not a frequent cause of late-onset ataxia of unknown origin, but should be considered in the cases with sensory neuropathy and ophthalmoplegia.
The study was supported by the NCN project 2012/05/B/NZ2/01627 and EU POIG.02.01.00-14-059/09

PS06.59

ANALYSIS OF THE EXPRESSION OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS GAMMA (PPAR GAMMA) AND miR-27 IN OBESE PATIENTS

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Obesity acts as a stress signal that induces multiple inflammatory responses in tissue, which plays an important role in the pathogenesis of cardiovascular disease and metabolic syndrome. The PPARgamma is a nuclear receptor that regulates adiposity differentiation, insulin sensitivity and lipid metabolism. Recent studies have shown that PPAR-γ may be regulated by miR-27a and miR-27b, pointing to a possible relationship with the development of obesity.

We compared 43 obese adults with body mass index (BMI) >35 who underwent bariatric surgery versus 19 healthy controls with BMI <25. Intraoperative biopsies of visceral fat and peripheral fat were taken. The expression of mRNA was examined by quantitative PCR analysis using a 7900 Fast Real-Time PCR machine. Statistical analysis was calculated by using the program SPSS.
Significant association was detected between a decrease in the expression of PPAR-γ1 in obese female patients and controls (P=0.045). We observed a negative correlation between expression of PPAR-γ1 in visceral adipose tissue, weight (P=0.016) and BMI (P=0.001). This inverse correlation was stronger in women group in which an inverse correlation of PPAR-γ2 was observed with weight (P=0.004) and BMI (P=0.001). No association was found in the expression of micro-RNAs in the group of cases and controls.
These findings suggest that obese patients with higher BMI have a lower expression of PPAR-γ1 in adipose tissue, particularly in women and visceral adipose tissue. The expression of PPAR-γ could be related to the metabolic status and the degree of obesity.
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PM06.60
A case of neonatal progeroid syndrome: Widemann-Rautenstrauch or Petty-Laxova-Wiedemann ?

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Progeroid syndromes are a group or rare genetic disorders that mimic physiological aging. Some of the syndromes in this group are presented at birth and therefore are called neonatal progeroid syndromes. Many of those syndromes have clear genetic causes still both neonatal progeroid syndromes of Wiedemann-Rautenstrauch and Petty-Laxova-Wiedemann, remain with unknown causes.
We report a 9 month old boy with progeroid features presented at birth. The physical examination showed marmorated and loose skin, apparent macrocephally, sparse hair, prominent scalp veins, small and beak shaped nose, downslating papebral fissures, triangular face, dysplastic, low set ears with small lobes, sharp and pointed chin, thin squamous toe nails.
Our patient presents features of both Wiedemann-Rautenstrauch and Petty-Laxova-Wiedemann. Most progeroid syndromes which are part of the differential diagnosis were rejected with DNA analysis. Additional laboratory findings, follow-up and management will be further discussed.

PS06.61
Genotype-Phenotype Correlation in PIK3CA-related overgrowth

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Introduction: *PIK3CA* encodes the p110α catalytic subunit of phosphoinositide 3-kinase and is a key mediator of cellular growth, survival and metabolism. Mosaic activating mutations in *PIK3CA* give rise to a wide spectrum of clinical presentations which are critically dependent upon the timing of the mutation during embryogenesis, and the cell type affected. The extent of genotype-phenotype correlation has been less clear from studies to date.
Method: DNA derived from affected tissue of 96 probands was target-enriched with an Ion AmpliSeq™ panel, and sequenced on an Ion Torrent platform. Clinical diagnoses at presentation included hemihyperplasia (25%), macrodactyly (16%), MCAP/MPPH (13%), and CLOVES (8%). 48/96 (50%) probands to date have been found to have mosaic *PIK3CA* mutations. Genotype-phenotype correlation analyses were performed using Chi-squared testing to compare clinical features and mutation site grouped by p110α functional domain (ABD, C2, helical and kinase).
Results: The only significant correlation identified was between genotype and the presence of vascular abnormalities, with higher proportions of patients with ABD and C2 domain mutations bearing vascular abnormalities.

Clinical feature(s)	% of probands with clinical feature	Correlation with *PIK3CA* Domain Mutated	Chi-squared P value
Progressive disease	56	No	0.63
Syndactyly	19	No	0.22
Polydactyly	8	No	0.30
Epidermal naevi	15	No	0.53
Scoliosis	13	No	0.16
Lipoma/lipomatous mass development	13	No	0.54
Vascular abnormalities	25	Correlated with ABD and C2 domain mutations	0.001

Conclusions: This small analysis suggests that *PIK3CA* genotype may indeed influence clinical presentation. Larger numbers of genotype-phenotype correlations should be undertaken to clarify this, to allow improved prognostication and patient stratification for clinical trials.
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PM06.62
Vitamin E therapy in Smith-Lemli-Opitz syndrome patients

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Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive, multiple malformation syndrome caused by 7-dehydrocholesterol reductase enzyme deficiency. Decreased cholesterol, elevated 7-dehydrocholesterol (7-DHC) and oxidative derivatives of 7-DHC (oxysterols) are thought to contribute to the pathophysiology of SLOS. According to recent data vitamin E could be effective in reducing oxidative stress in SLOS patients. We have started vitamin E treatment (in addition to cholesterol supplementation) in seven SLOS patients. Starting daily doses of RRR-alpha-tocopheryl acetate were 230 mg for young patients (age group: 4 to 10 years) and 2x230 mg above 10 years of age. Plasma vitamin A and E concentrations of the patients were monitored monthly by HPLC method. Behavioral effects of the treatment were determined using the Aberrant Behavior Checklist (ABC). All patients had vitamin E deficiency before the treatment, while vitamin A concentrations were normal in all but one patient. Vitamin E levels reached reference range in five patients after one month and remained stable. According to four scores (irritability, lethargy, stereotypic behavior, hyperactivity) of ABC no positive effect on behavior could be detected after 3 months. The fact that our patients were vitamin E deficient but vitamin A concentrations were in the normal range except for one patient suggests a higher utilization of vitamin E and not an inadeqaute absorption. No positive behavioral effects of the treatment could be detected after the first three months.
This study was supported by the Hungarian Research Fund (K109076, I.B.).

PS06.63
Morphological and proteomic studies reveal autophagy as a main driver on Smith-Lemli-Opitz syndrome

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Introduction: Studies reporting the dramatic consequences of low cholesterol levels on cells are scarce. In this study we used fibroblasts from Smith-Lemli-Opitz syndrome patients (SLOS) - a metabolic genetic disease affecting the cholesterol biosynthesis pathway - to investigate the consequences of cholesterol deficiency on cell morphology and protein expression.
Materials and methods : Morphological studies (MTT test, immunocytochemistry for LC3, MDC and acridine orange coloration as well as electron microscopy) and proteomic analysis (iTRAQ LC /MS-MS) were performed on fibroblasts from SLOS patients and human controls, simultaneously cultivated both on standard conditions and cholesterol depleted media.
Results: Morphological studies showed that when endogenous synthesis of cholesterol is inadequate (SLOS) and there is no appropriate supply to overcome cellular needs (cholesterol depleted media), cell proliferation in vitro becomes impaired and autophagy is activated.
Furthermore, SLOS cells in cholesterol depleted medium show an overexpression of a set of proteins. Mainly, these cells seems to increase MnSOD expression to combat oxidative stress derived from the increased amount of 7-dehydrocholesterol and its derivatives, caused by the inherited enzymatic deficiency and thus control cell proliferation, whereas heat shock 70 kDa protein 4, an autophagic protein (Atg2) also presents a cytoprotective activity and inhibits apoptosis.
Conclusion: We conclude that the mechanism by which SLOS fibroblasts handles their metabolic deficit, involves autophagy which plays an important role in cell survival. Furthermore this work provided powerful indications that may be useful to expand the knowledge about the mechanisms involved in cellular pathophysiology of SLOS.

PM06.64

Association of GLP1R and HTR2A Polymorphisms with Obesity and Metabolic Traits in a Malaysian Population

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Obesity is frequently associated with metabolic complications such as hypertension, diabetes and cardiovascular diseases. Obesity is a complex disease with high heritability estimates (> 0.70). Glucagon-like-Peptide-1 receptor (GL1PR) plays important roles in glucose homeostasis and inhibition of appetite by acting on brain. On the other hand, Serotonin Receptor (HTR2A) is a neurotransmitter which works closely with leptin and insulin in metabolic response to dietary intake. It is largely unclear whether genetic variations in these receptor genes lead to individual differences in susceptibility to metabolic complications. Therefore we investigated the associations of single nucleotide polymorphisms (SNPs) of GL1PR and HTR2A genes with obesity and metabolic traits. A total of 676 Malaysian Malays, obese (n=197) and non-obese (n=479) were genotyped for the GL1PR rs2268641 and HTR2A rs912127 SNPs. Anthropometric, fasting plasma glucose, body fat and lipid levels were measured. Data were analyzed using Multivariate linear models. After adjusting the age and gender, it was found that GL1PR rs2268641 was significantly associated with hip circumference (HC), (p=0.015). The GA allele carriers had significantly higher HC (Mean (cm) =102.22±9.83) compared to GG and AA homozygotes. HTR2A rs912127 SNP was also significantly associated with waist hip ratio (WHR), (p=0.001) after adjusting the age and gender. AA homozygotes had higher WHR (Mean=0.89±0.10) compared to G allele carriers. However, both the SNPs were not associated with logBMI. While these findings require replication in larger studies, we provide new evidence that GLP1R and HTR2A receptor gene variants may involve in metabolic abnormalities in Malaysian Malays.

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PS06.65

Exome sequencing identifies TMEM70 deficiency in a Hungarian Roma family with severe congenital lactic acidosis

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Pyruvate dehydrogenase deficiency underlies over half the cases of congenital lactic acidosis in children of Roma ethnicity, while lactic acidosis is also a presenting symptom of various rare mitochondrial disorders. We report here a Hungarian Roma family with two boys affected by severe congenital lactic acidosis, poor feeding, lethargy, cardiomyopathy, hypotonia and respiratory failure. The first boy died at 6 months of age due to sepsis and acute metabolic crisis, while the second boy survived with early initiation of a ketogenic diet and shows severe developmental delay, hypotonia, growth retardation, microcephaly and cardiomyopathy at 2,5 years. Enzymatic analysis of patient fibroblasts did not reveal any abnormality in pyruvate dehydrogenase activity, and no mtDNA mutations were detected. Whole exome sequencing (WES) of the second son with SureSelect Human All Exon Kit and 100 basepair paired end sequencing was carried out on an Illumina HiSeq2500 system with >97% of target covered >20-fold. Applying an autosomal recessive model, 94 rare variants were identified, but only 3 out of 20 annotated genes were known to have a role in mitochondrial metabolism. Only *TMEM70* carried a homozygous predicted loss-of-function variant: the common c.317-2A>G splice site mutation resulting in ATP synthase deficiency. Segregation analysis revealed homozygosity in both affected brothers and heterozygosity in the parents and the healthy sister. We describe the first Hungarian family with TMEM70 deficiency, supporting the clinical utility of WES when addressing a group of disorders with large genetic heterogeneity.

OTKA **103983 BM,** OTKA **PD 112831 KK**, Janos **Bolyai Research Scholarship KK, JB**

PM06.66

Thioredoxin 2 deficiency causes early-onset neurodegeneration

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Thioredoxin 2 (TXN2) is a mitochondrial redox protein, which is ubiquitously expressed with the highest expression levels in brain tissue. It is encoded by a nuclear gene, *TXN2*, containing a mitochondrial targeting sequence. TXN2 plays a crucial role in oxidative stress defense and in the regulation of the mitochondrial apoptotic pathway. Animal studies suggest that TXN2 is essential during embryonic development.

Using exome sequencing in a 15-year-old adolescent suffering from an early onset neurodegenerative disorder with severe cerebellar atrophy, epilepsy, peripheral neuropathy, dystonia and combined respiratory chain deficiency in muscle tissue, we identified a homozygous stop mutation in *TXN2*. TXN2 protein was not detectable in patient fibroblasts, confirming the predicted loss of function. Cellular studies revealed increased reactive oxygen species (ROS) levels, impaired oxidative stress defence and secondary mitochondrial dysfunction with reduced cellular respiration and diminished ATP production. Lentiviral expression of TXN2 restored all these parameters confirming the causal role of the mutation. Supplementations with antioxidants effectively suppressed cellular ROS production, and lead to moderate clinical improvement during short term follow-up of the patient.

Our report on the first patient with TXN2 deficiency highlights the importance of TXN2 for neurodevelopment. Moreover, our results point to a potential role of antioxidant treatment in affected patients.

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PS06.67

A Patient with Ornithine Transcarbamylase Deficiency treated with Glycerol Phenylbutyrate: Preliminary Experience

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Control of hyperammonemic crisis in patients with Urea Cycle Disorders (UCDs) is of utmost importance to preserve cognition.

Glycerol phenylbutyrate (GPB) is an oral liquid formulation that has been recently approved by FDA for chronic management of patients >2 years old with UCDs that cannot be adequately managed by a protein- restricted diet. It has the same mechanism of action as Sodium phenylbutyrate NaPBA, but it is a sodium- and sugar-free pre-pro-drug of phenylacetic acid (PAA) that has little odor and taste.

Here we report a 13-year old OTC female with moderate cognitive delay, hyperactivity and recurrent hyperammonemic crisis (every 1-2 months). The patient had poor compliance to NaPBA due to it is salty taste and peculiar odor. After starting GPB, the patient showed significant improvement in the compliance as the medication was more tolerable compared to NaPBA. Her mother reported full compliance with the drug. Since starting the medication, there was significant decrease in ER visit/admissions with subjective improvement in activity.

Several short-term randomized controlled trials in adults and children with UCDs have supported the effectiveness of GPB in long term control of hyperammonemia, as well as significant improvement of behavioral function of UCDs patients. [1-5]

This is a preliminary report that supports the accumulating evidence of the effectiveness of this medication as a long term alternative choice for NaPBA non-complaint Urea Cycle patients.

PM06.68

Not typical North-European ATP7B gene mutation in Latvian patients with Wilson disease

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Introduction.

Wilson disease (WD) is a disorder of copper metabolism, caused by mutations in gene *ATP7B*. More than 500 distinct mutations have been described in the *ATP7B* gene, from which 380 have a confirmed role in the pathogenesis of the disease (Wilson disease mutation database: http://www.wilsondisease.med.ualberta.ca/database.asp.). The most common mutation in the

Europe is H1069Q.
Materials and methods.
In total 235 patients have been sent for WD DNA diagnostics in Latvia. All the patients were first tested for the most common mutation H1069Q, by using PCR Bi-PASA method; 64 patients, clinically confirmed with WD, where the mutation H1069Q was not be found in both alleles, were tested for other possible mutations (in the exons 2, 3, 5, 6, 8, 11, 13, 14, 16, 18 of gene ATP7B – exons having the most common prevalence of mutations in WD patients) by direct sequencing.
Results.
Wilson disease was genetically confirmed in 42 patients. In total 68.94% of WD patients' alleles were identified: H1069Q (62.12%); M769Hfs*26 (2.27%); Val73GlufsX4 (1.52%) and D1267G, D765N, M645R, V1036I (0.76% for each); 31.06% of the alleles remained unidentified. Among the patients silent or not disease causing variants were found: R725R (2.27%); A1140V (12.12%); S406A (18.94%).
Conclusions.
1) The most prevalent mutation found in Latvian patients is H1069Q – in 62.12% cases;
2) Mutation Val73GlufsX4 presented in two patients is described among Sardinians and Bulgarians;
3) The study should be continued to test all the ATP7B gene, since the selective screening has not identified all the mutations.

PS06.69
Identification of a new CISD2 mutation in a French patient with a typical Wolfram syndrome

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Wolfram syndrome is an autosomal recessive disorder characterized by diabetes mellitus and optic atrophy usually associated with diabetes insipidus, deafness, renal tract abnormalities or neuropsychiatric disorders, mainly associated with mutations in the WFS1 gene. Mutations in a second gene, CISD2, have also been described in patients with Wolfram syndrome type 2 characterized by diabetes mellitus and optic atrophy associated with peptic ulcer disease, bleeding tendency but without diabetes insipidus and psychiatric disorders. To date, only five families have been described and few data are available concerning both clinical phenotype and CISD2 function.
Among our French Wolfram syndrome cohort of 98 patients with at least diabetes mellitus and optic atrophy, we identified 2 WFS1 mutations in 78 patients (80%). We sequenced the CISD2 gene in the 20 patients with only one WFS1 mutation (7/98 patients, 7%) or without any mutation (13/98 patients, 13%). We identified a new missense homozygous CISD2 mutation, c.215A>G (p.Asn72Ser), in a patient with a «classical» Wolfram presentation without digestive or hematologic symptoms and who did not carry any WFS1 mutation. Segregation within the family, in silico analysis and absence in control individuals are in favor of the pathogenicity of this new variant.
In conclusion, we show that CISD2 must be analyzed in patients presenting with a «classical» Wolfram syndrome when WFS1 has been ruled out. Functional studies on primary fibroblasts, carrying the p.Asn72Ser CISD2 mutation, are in progress to determine the effects of this mutant and to understand the link between wolframin and CISD2 proteins.

PM06.70
Familial Chylomicronemia: Report of two Chinese infants presenting with lipemic plasma

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We used a monogenic dyslipidemias panel paralleled next generation sequencing assay to detect disease causing mutations in two infants presented with severe hypertriglyceridemia and lipemic plasma.
Case Report-Case A: A 30-day-old Chinese boy, who was admitted to the local hospital for coughing for ten days, and the blood was found to be pink. Fasting serum lipids, which included triglyceride (TG) and cholesterol (CHOL) were abnormal, total cholesterol (TC) and triglyceridemia (TG) was 1270 and 757mg/dL, respectively. Subsequent genetic testing of the patient revealed compound heterozygosity of p.Arg270His and p.Trp421* mutations on LPL gene, both of which were known pathogenic mutations. Low-fat/low-cholesterol diet therapy was introduced. Unexpectedly, serum cholesterol level decreased dramatically, and normalized in 2 months.
Case Report-Case B: A 48-day-old Chinese boy initially presented with milk choking and polypnea, but no other co-morbidities. His triglyceride (TG) was 557mg/dL, but the cholesterol (CHOL) was normal. Genetic testing of the patient revealed compound heterozygosis of two known pathogenic mutations: one is p.Leu279Arg and another is a large fragment deletion on exon8/exon9/exon10. With a diet of low fat and vitamins added, the TG was controlled in a good level, and the baby was developing well in regard to stature, weight, and mind.
Based on the regional hybrid capture parallel next generation sequencing platform, both SNVs and large fragment deletion could be identified simultaneously. Familial Chylomicronemia is characterized by very severe hypertriglyceridemia and will lead to acute pancreatitis, which is often life-threaten. Timely diagnose and prevention is critical for patients' management and recovery.

PS07.01
A missense SNP in CNR2 gene is associated with childhood asthma severity and treatment outcome

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Both the interaction of several genes as well as environmental factors influences the asthma pathogenesis. The rs2229579 is a missense SNP in the CNR2 gene that codes for the cannabinoid receptor 2.
We analyzed rs2229579 association with childhood asthma, and the effect of rs2229579 on the response to inhaled corticosteroids (ICS) or leukotriene receptor antagonist (LTRA) treatment and CNR2 gene expression. We studied a case-control cohort of 341 children mild/moderate persistent asthma and 245 controls. Blood samples were collected before treatment, and 4-6 weeks after treatment with ICS or LTRA.
Atopic asthmatic patients with CT genotype presented significantly higher FENO ($p=0.0303$), total IgE ($p=0.0146$) and lower log PC20 ($p=0.0403$). Non-atopic asthmatics with CT presented only significantly higher eosinophil count ($p<0.001$). After ICS treatment atopic asthmatics with CT genotype had less improvement of condition as measured by dFEV1/FVC ($p=0.0440$), while after LTRA treatment asthmatics with CT genotype had worsening condition measured by dFEV1/FVC ($p=0.0286$). No association was found between rs2229579 genotype and asthma or CNR2 gene expression. We found a significantly increased CNR2 gene expression in asthmatics before treatment compared to healthy controls ($p<0.0001$). Higher CNR2 gene expression was correlated with more severe asthma measured by FEV1/FVC ($p=0.0160$).
Our results suggest rs2229579 is associated with asthma severity and with ICS or LTRA treatment response in children with asthma, and suggest the involvement of the endocannabinoid system in asthma.
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PM07.02
Association of NCOA5 gene rs2903908 variant with the Behcet's disease

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Abstract
Behçet's disease (BD) is a multisystemic chronic inflammatory autoimmune disease of unknown etiology. Various genetic and environmental factors have effects on the pathogenesis of the Behcet's disease. NCOA5 gene encodes coregulator protein for ER-α which have affect in regulating autoimmunity, may have an impact on BD pathogenesis.
In this study, we aimed to clarify the impacts of the NCOA5 rs2903908 single nucleotide polymorphism (SNP) on susceptibility and clinical findings of BD.
This study included 300 BD patients and 288 healthy control patients. Genetic analyses were performed by using the TaqMan allelic discrimination assay. SPSS 16.0 Software was utilized to estimate OR and Chi-square tests.
The TT genotype of the NCOA5 rs2903908 SNP were significantly higher in

overall BD patients compared to those in healthy controls, and in patients with female gender compared to healthy female controls (p = 0.01, and p = 0.026, respectively). The CT genotype of the NCOA5 rs2903908 SNP was significantly higher in the patients with BD who had genital ulceration or uveitis compared to those in the patients group without genital ulceration and uveitis (p = 0.002, and p = 0.005, respectively). The C allele frequency was found statistically significantly higher in the BD patients with uveitis compared to those in the patients group who did not have uveitis (p = 0.0001, OR, 95%CI = 2.19, 1.48-3.23).
Our study represents the first time that the NCOA5 rs2903908 SNP seemed to be linked to BD susceptibility and clinical findings.

PS07.03
Frequency of the CCR5-delta 32 chemokine receptor gene mutation in the Czech population - study of 1 385 individuals

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Introduction: A direct correlation between HIV infection and mutation in the chemokine receptor (CCR5) gene has been established. The 32 bp deletion in the gene (CCR5-delta 32) alter function of the protein product, thereby altering chemokine binding/signaling. Homozygous carriers of the CCR5-delta 32 mutation are resistant to HIV-1 infection because the mutation prevents functional expression of the CCR5 chemokine receptor normally used by the most of HIV-1 strains to enter CD4 T cells. However, resistance is not absolute due to alternative CXCR4 co-receptor and HIV-1 isotropic switch.
Materials and methods: In the present study, we have determined by PCR the allelic frequency of the CCR5-delta 32 mutation in 1385 Czech individuals aged 4 - 86. CCR5-delta 32 homozygotes were confirmed by sequencing.
Results: 18,8% of studied individuals were heterozygous and 1,2% were homozygous for the mutation. Overall, the frequency for the CCR5-delta 32 allele was 10,6%. Distribution of the mutation was unaffected by sex or age.
Conclusion: The mutation is found principally in Europe and western Asia, with higher frequencies generally in the north. The frequency in the Czech population population is consistent with the origin of the mutation in northern Europe and natural gene flow into the central Europe. The CCR5-delta 32 mutation is a good example of an advantageous allele with a well-characterized geographic distribution, which may play important role in managing of HIV infection in the future. Allogeneic stem-cell transplantation or genome engineering represent the most promising HIV treatment options.

PM07.04
Expression analysis of Toll-like receptor pathway in celiac disease

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Introduction: Celiac disease (CD) is a chronic, immune-mediated gastrointestinal disorder that develops in genetically susceptible individuals due to an inappropriate immune response to ingested gluten. The only available treatment is a life-long gluten-free diet (GFD). Toll-like receptor (TLR) pathway is known to be crucial in the regulation of innate immunity, but also participates in the development of antigen-specific adaptive immunity. The aim of this study was to characterize a possible deregulation in TLR pathway in CD.
Materials and Methods: The expression of 28 genes related to TLR pathway was measured by RT-PCR in biopsies from 16 patients with active CD, 16 patients in a GFD and 15 non-celiac controls. The three groups where compared.
Results: Nine of the 28 genes were differentially expressed in active patients when compared to controls. *CXCL11*, *IFNG*, *CXCL10*, *IL10*, *IL6* and *CCL5* were overexpressed, whereas *TICAM1*, *TOLLIP* and *IRF3* were underexpressed. When treated patients and controls were compared, *IRF5* was underexpressed, and *TIRAP* overexpressed. *CXCL11*, *IFNG*, *CXCL10* and *IL6* were differentially expressed between active and treated patients. Results were contextualized with the results of 23 additional genes obtained recently in our laboratory.
Conclusions: Our results confirmed significant alterations in several genes. Patients treated with GFD showed a less altered pattern, although some genes remain altered suggesting a constitutive role in the pathogenesis of CD. Additionally, different degrees of alteration among the receptors, the pathway core and genes encoding costimulatory proteins, cytokines and interferons in active patients suggest a probable alteration downstream of the receptors.

PS07.05
Rapid detection of the three celiac disease risk genotypes HLA-DQ2.2, DQ2.5 and DQ8 by multiplex ligation dependent probe amplification.

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Celiac Disease (CD) is a chronic, small intestinal enteropathy and is associated with the HLA genotypes HLA-DQ2.5 (HLA-DQA1*05 / HLA-DQB1*02), HLA-DQ8 (HLA-DQA1*03 / HLA-DQB1*0302). More recently also HLA-DQ2.2 (HLA-DQA1*02 / HLA-DQB1*02) has been identified to predispose for CD. Genotyping of the HLA-DQA1 and HLA-DQB1 genes is important since genetic HLA-DQ2.2, -DQ2.5, and -DQ8 testing has a very strong negative predictive value. Testing for relevant alleles is therefore recommended for risk stratification of individuals genetically susceptible to CD.
The Multiplex Ligation-Dependent Probe Amplification (MLPA) probemix P438-D1 was developed to simultaneously detect the A and B subunits of the heterodimeric DQ2.2, DQ2.5, and DQ8 molecules. For each allele, i.e. HLA-DQA1*02, HLA-DQA1*03, HLA-DQA1*05, and HLA-DQB1*02, two probes have been added to the probemix. Three probes have been included to detect the HLA-DQB1*0302 allele of the HLA-DQ8 B-unit: one probe detects all HLA-DQB1*03 alleles while the other two probes detect HLA-DQB1*0302/*0303 or HLA-DQB1*0302/*0305.
Results obtained by the MLPA assay confirmed the results of 39 samples previously genotyped by conventional PCR-SSO, PCR-SSP and PCR-SSCP/HD methods, to accurately determine the presence of DQ2.2, DQ2.5 or DQ8 genotypes. In addition, MLPA has been shown to be able to distinguish between heterozygous or homozygous state of the alleles which is of importance since homozygous HLA-DQB1*02 in presence or absence of HLA-DQA1*02 or HLA-DQA1*05 also predisposes for CD.
The P438-D1 MLPA probemix provides a quick, reliable and complete method to determine all three HLA-DQ genotypes relevant for CD.

PM07.06
Frequencies of haplotypes associated with Coeliac disease in the Czech population

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Celiac disease (CD) is a chronic small intestinal immune-mediated enteropathy precipitated by exposure to dietary gluten in genetically predisposed individuals. There are HLA-DQ heterodimers that especially increase an individual's predisposition to CD: DQA1*0501-DQB1*0201 in cis position (haplotype DQ2.5cis), DQA1*0505 - DQB1*0301/ DQA1*0201 - DQB1*0202 in trans position (haplotype DQ2.5trans) and DQA1*0301-DQB1*0302 in cis positon (haplotype DQ8.1). The aim of this study was to determine the frequency of mentioned CD-risk haplotypes in Czech population and to compare it with the previously published data.
216 healthy individuals (101 males and 115 females, age 18-69 years) of the Czech population were tested. DNA was isolated from oral mucosa or blood using MagCore HF16. CD-risk alleles were tested by strip assay certified method (CeliacStrip).
The overall frequency of CD-risk haplotypes in Czech population was 36.1%. Haplotype DQ2.5cis was detected in 18.5%, haplotype DQ8.1 in 14.8% and haplotype DQ2.5trans in 2.8% of samples. In 30.6% of samples no risk allele was detected.
The frequency and dominance of DQ2.5cis over DQ2.5trans haplotype is in agreement with previously published data, but the high frequency of DQ8.1 among individuals with genetic risk (41%) is surprising, because this haplotype is found only in 5% of patients with CD in Europe. This may indicate that DQ8.1 is among Czech patients with CD in higher frequency than in European or DQ8.1 haplotype means lower genetic risk to develop CD or it is associated with subclinical CD (non-typical or silent form) and patients with this haplotype are not diagnosed.

PS07.07
Genome-wide copy number variation analysis in systemic lupus erythematosus

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Systemic Lupus Erythematosus (SLE) is an autoimmune disease with a strong genetic background characterized by chronic inflammation and autoantibody production. Genome-wide association studies identified several loci associated with SLE pathogenesis. However, there are few studies that focus on the role of copy number variations (CNVs) in SLE etiology. The purpose of this study was to determine the role of CNVs in SLE patients. Lupus nephritis (LN) was a criterion of inclusion for this study. Blood cell lymphocytes from 23 SLE unrelated patients and 44 healthy subjects were submitted to the Genome-Wide Human Cytoscan HD Array (Affymetrix®) in order to screen for CNVs in genomic DNA. SLE patients and controls had no statistic difference in total CNV numbers, median size of CNVs, and the proportion of the genome covered by CNVs. However, our results showed that SLE patients have common and rare CNVs in the loci that may contribute to the increased risk for SLE. We identified six common copy number variation regions (CNVRs) as potential candidates involved with SLE in 8p11.22, 14q32.33, 22q13.33, Xp22.33 (2), and Xp26.2 loci (OR = 3.2–11.94; p = 0.0002–0.033). A total of 147 rare CNVs were detected and validated *in silico* in SLE patients. Rare deletions were found in two genes related to autoimmunity: *STAT4* and *CFHR5*. This is the first report describing CNVs and CNVRs encompassing these regions in SLE patients. Financial Support: FAPESP (2013/17062-9;2011/23794-7), CNPq (312547/2009-9) and CAPES.

PS07.09

Whole-exome sequencing of common variable immunodeficiency

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Introduction: Common variable immunodeficiency (CVID) is the most common symptomatic primary immunodeficiency, with a prevalence between 1/ 30,000 to 1/200,000 and reported incidence of 1/75,000 births. The genetic defect underlying some of the primary immune deficiencies including CVID remains unknown, and the current hypothesis is that it might be multigenic. We have applied the next-generation sequencing technologies to the study of CVID.
Material and Methods: The study has been performed in 36 patients with CVID diagnosed from a Spanish cohort including sporadic as well as familiar cases. CVID diagnosis was made using current international criteria. Patients were analyzed by exome sequencing and copy number variants (CNV) analysis.
Results: We obtained an initial list of about fifty candidate genes for each patient. Candidate genetic variants were validated in the context of pedigrees, according to different genetic models for the disease. Functional classifications coupled with an in-house algorithm for prioritizing mutations generated a shorter list of candidate genes for each patient. The integrated analysis of genetic data, together with the collected clinical and laboratory data, narrowed down the list of candidate causal genes and mutations. Functional validation of candidate genes is being performed to test their role in the molecular origin of CVID.
Conclusions: Exome sequencing is a powerful tool to detect the causal variants in CVID cases with Mendelian inheritance. Our results also suggest an oligogenic model for the disease in some patients.
Funding: This work was funded by grant SAF2012-35025 from the Spanish Ministerio de Economía y Competitividad to FC.

PM07.10

Sanger sequencing of neutrophil elastase in a neonate with congenital neutropenia

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Introduction: A male neonate presented with recurrent deep-seated skin infections within the first four weeks of life. Full blood count identified severe neutropenia. Separation of the umbilical cord was delayed beyond two weeks of life; physical examination of the neonate was otherwise normal with no dysmorphic features identified. Family pedigree was unremarkable.
Methods: Sanger sequencing of the ELANE gene was performed in the proband, mother and father. Sequencing was approved by the NHS National Research Ethics Service (12/SC/0044) with the purpose of accurate diagnosis prior to bone marrow transplant.
Results: Sequencing identified a single heterozygous missense mutation in Exon 2, not present in either parent. The mutation (c.193G>T) was predicted to result in an amino acid change p.(Val65Phe). This would alter the property of the specific amino acid from an aliphatic Valine to an aromatic Phenylalanine. SIFT analysis predicted this variant to affect protein function with deleterious effects that would not be tolerated (calculated probability of 0.00). Polyphen 2 analysis predicted this variant to be highly damaging (calculated probability of 1.00).
Conclusions: This is the first description of delayed separation of the umbilical cord associated with an ELANE c.193G>T mutation. Neonatal neutropenia due to a variety of causes is associated with delayed separation of the cord. Although the c.193G>T mutation has previously been reported in cases of congenital neutropenia, more detailed clinical phenotyping of these rare cases will help reduce time to diagnosis in this life-threatening and potentially treatable disease. The baby requires high doses of G-CSF (25 mcg/kg/day), which is in keeping with a severe phenotype.

PS07.11

Courier gene to deliver embedded DNA vaccine to immunize patients against intracellular human immunodeficiency virus

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A DNA sequence has been designed to be embedded into the human genome (Table 1), when transcribed is intended to generate a mRNA, this mRNA when translated to produce a therapeutic Transcription Factor IIIA (TFIIIA) molecule to engage the HIV genome (GenBank K03455.1). We modified TFIIIA molecule (NC_000013), altering zinc fingers 1-5, to bond to HIV's unique identifier, ‚agcagctgctttttgcctgtactgg', nucleotides 431-455, the 25 nucleotides located between the TATA box and transcription start site. We studied amino acid-nucleotide binding characteristics. Permanently binding a TFIIIA to HIV's unique identifier is intended to prevent transcription of the HIV genome. Using a reverse transcription process, we developed a transcribable segment of DNA. The Tumor Necrosis Factor alpha (TNF) gene (AY214167.1) is active in CD4+ lymphocytes, including the reservoir for HIV. Transcribable sequence of DNA coding for the therapeutic TFIIIA molecule replaces the TNF's original mRNA's protein coding sequence from nucleotides 1750-4519. The modified TNF gene utilizes TNF gene's original 5' upstream and 3' downstream signaling segments. The modified TNF gene acts as a courier to transport the embedded DNA vaccine sequence. Likened to HIV genome, the modified TNF gene is inserted into the human genome and functions like an alternative copy of the TNF gene. The embedded DNA vaccine undergoes transcription, to produce mRNAs which generate TFIIIAs designed to seek out and silence the HIV genome, creating a cell defensible against intracellular HIV infection.
Table 1. Embedded DNA vaccine

Sequences	5' Upstream TNF alpha gene sequence AY214167.1	TFIIIA Zinc Fingers	Modified TFIIIA gene sequence (as Amino Acids) NC_000013	3' Downstream TNF alpha gene sequence AY214167.1
Original:	1-1749	---	1-11166	4520-7240
Modified:	1-1749	---	1750-12915	12916-15636
---	---	---	---	---
---	----------	1-Original:	336-371	----------
---	----------	Sequence:	SANYSKAWKLDA	----------
---	----------	1-Modified:	2085-2120	----------
---	----------	Coding:	NSSRESSNSSRE	----------
---	----------	2-Original:	2579-2614	----------
---	----------	Sequence:	GKAFIRDYHLSR	----------
---	----------	2-Modified:	4328-4363	----------
---	----------	Coding:	KSSRESSKSSKK	----------
---	----------	3-Original:	5346-5381	----------
---	----------	Sequence:	DQKFNTKSNLKK	----------
---	----------	3-Modified:	7095-7130	----------
---	----------	Coding:	KSSKRSSESSEK	----------
---	----------	4-Original:	6008-6043	----------
---	----------	Sequence:	KKTFKKHQQLKI	----------
---	----------	4-Modified:	7757-7792	----------
---	----------	Coding:	RSSKNSSESSKR	----------
---	----------	5-Original:	8207-8242	----------
---	----------	Sequence:	GKHFASPSKLKR	----------
---	----------	5-Modified:	9956-9991	----------
---	----------	Coding:	RSSRKSSESSKE	----------

PM07.12

A DGKE intronic mutation explains genetically unsolved cases of familial atypical hemolytic uremic syndrome

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Genetic and acquired abnormalities causing dysregulation of the complement alternative pathway contribute to atypical hemolytic uremic syndrome (aHUS), a rare disorder characterized by thrombocytopenia, non-immune microangiopathic hemolytic anemia, and acute kidney failure. However, in a substantial proportion of patients the disease-associated alterations are still unknown.
We studied a family with infantile recessive aHUS, in whom whole-exome sequencing analysis with conventional variant filtering parameters did not reveal any obvious candidate mutation. The report of aHUS-associated mutations in *DGKE*, encoding diacylglycerol kinase epsilon, led us to re-examine the non-coding *DGKE* variants obtained from next-generation sequencing, allowing identification of a novel intronic *DGKE* mutation (c.888+40A>G) that segregated with disease. Sequencing of cDNA from affected individuals revealed aberrant forms of *DGKE* mRNA predicted to cause profound abnormalities in the protein catalytic site. The same mutation was found in compound heterozygosity with a second nonsense *DGKE* mutation in all affected siblings of another unrelated family. Homozygous and compound heterozygous patients presented similar clinical features, including aHUS presentation in the first year of life, multiple relapsing episodes and proteinuria, which are prototypical of DGKE-associated aHUS.
This is the first report of a mutation located beyond the exon-intron boundaries in aHUS. Intronic mutations such as these are underreported since conventional filtering parameters used to process next-generation sequencing data routinely exclude these regions from downstream analyses in both research and clinical settings. Our results suggest that analysis of non-coding regions of aHUS-associated genes coupled with mRNA sequencing might provide a tool to explain genetically unsolved aHUS cases.

PS07.13

Clinical exome sequencing of Finnish primary immunodeficiencies

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Primary immunodeficiency diseases (PIDs) are disorders in which part of the immune system malfunctions as a result of mutations in immune related genes. To date, over 220 genes responsible for these diseases have been recognized, and this list is rapidly growing. The diagnosis of PIDs based on symptoms is often difficult, as they overlap between disorders and can also vary considerably between individuals with mutations in the same gene. To elucidate the genetic causes for disease, we are performing clinical whole exome sequencing on Finnish PID patients. Our cohort currently consists of roughly 150 index cases, about 100 of which have been sequenced by now. We have detected both previously identified and novel disease variants in known PID genes, as well as identified putative disease variants in genes that have not been previously associated with such diseases. For example, several variants in the *TNFRSF13B* gene have been previously described as predisposing to CVID, and interrogation of the exome sequence data of Finnish population cohorts (Sequencing Initiative Suomi) shows that some of these variants are enriched in Finland. This could partially explain why CVIDs seem to be overrepresented in Finland compared to the rest of Europe. Consistently with this, we have identified previously reported disease variants in *TNFRSF13B* in three patients with CVID. However, several of the sequenced CVID patients do not carry mutations in this or any other previously reported PID gene, and thus warrant further investigation.

PM07.14

Mapping of private variations in human MHC conserved extended haplotypes

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Distinct long-range genetic fixation in the MHC region, known as conserved extended haplotypes (CEHs), have been described in numerous studies due to its unique genomic characteristic and strong association with many complex traits. However, despite the great interests, the limitations of technology and heterogeneous nature of MHC haplotypes have thus far prevented extensive study of sequence variation in CEHs. As such, the level of intra-CEH polymorphism and the possible effect of these variants are not well-understood. In the study, with the use of multiple MHC homozygous cell lines, we demonstrated extensive sequence conservation in two common MHC CEHs of Asian ancestry - A33-B58-DR3 and A2-B46-DR9 by high throughput genome sequencing. This approach has allowed us to assemble at least 90% phase-resolved MHC sequence representative of the A33-B58-DR3 and A2-B46-DR9 haplotype. We also for the first time described the extent of intra-CEH variation within the conserved boundaries of the MHC CEHs and revealed that the level of variation is non-homogenous across the MHC region. Importantly, our study showed evidence that these private intra-CEH variants could potentially have functional impact in the context of gene expression. The availability of these alternate Asian MHC sequences would complement the eight European MHC haplotype sequenced by the MHC Haplotype Project and provides a framework to study the MHC diversity and variations. This insight gained from the results of this study may also enable an improved approach to MHC genetic dissection of disease-causing variants and genes.

PS07.15

WRN helicase plays an important role in multiple myeloma proliferation and inhibition of its activity by NSC19630 induces cytotocixity

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Multiple myeloma (MM) is a plasma cell malignancy characterized by proliferation of monoclonal plasma cells in the bone marrow. Despite several therapeutic approaches, including high-dose chemotherapy, stem cell transplantation and the usage of novel agents such as bortezomib, MM still remains an incurable disease. Extensive genomic instability associated with molecular heterogeneity is a major hallmark of MM cells. Unfaithful DNA repair has been implicated to promote the genomic instability and cancer cell survival. Werner Seyndrome (WRN) helicase is a member of RecQ helicase family and contributes to DNA replication, recombination and repair. The supression of WRN expression in cancer cells by siRNA or its inhibitor led to an increased succebility to agents generating DNA damage and cell death by stimulating apoptosis. Therefore, the inhibition of WRN expression might be a potential target for increasing the susceptibility of MM cells against chemotherapeutic drugs. In this study, we show the increased expression of WRN in both newly diagnosed and relapsed CD138+ myeloma plasma cells compared to both normal subjects and their CD138- non-tumorigenic cells. Furthermore, by using NSC19630 a specific WRN helicase inhibitor, we further show a decreased proliferation and an increased apoptosis in MM cells. These results show that WRN is involved in MM proliferation and its inhibition may serve to enhance the efficacy of chemotherapy.
This research has been supported by The Scientific and Technological Researc Council of Turkey (No:113Z383).

PM07.16

NLRP3 and MEFV gene variants in PFAPA patients in Slovenia

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PFAPA syndrome is the most common autoinflammatory fever disorder in childhood, with recurrent fever, aphthous stomatitis, pharyngitis and adenitis. Mutations in the MEFV and NLRP3 genes cause syndromes with PFAPA overlapping symptoms which are rarely reported in patients from Slovenia. The aim was to assess the frequency of MEFV and NLRP3 gene variants in patients with PFAPA syndrome in order to determine whether genes involved in other autoinflammatory diseases, might play a role in PFAPA pathogenesis.
We collected clinical and laboratory data of PFAPA patients under the age of 5, who were followed at the University Children's Hospital Ljubljana. All 10

exons of MEFV and 9 exons of NLRP3 genes were directly sequenced.
30 PFAPA patients were tested for MEFV and NLRP3 gene variants. Ten patients (33%) had 11 variants, all in heterozygous state. 6 patients have Q703K variant in NLRP3, one E148Q in MEFV and one combination of I591T in MEFV and Q703K in NLRP3. Novel variant in NLRP3, P200T, was identified in one patient. One girl was found to have known variant in NLRP3 S726G, which is associated with CINCA syndrome. She has had typical PFAPA symptoms, but also epilepsy and developmental delay. Five different gene variants were identified in 10/30 PFAPA patients with MEFV variants found in 2 patients and NLRP3 variants in 9. Our results indicate genetic heterogeneity of PFAPA population and overlap with other periodic fever syndromes.

PS07.17
Whole-exome sequencing (WES) for the identification of underlying genetic events in patients with primary immune deficiency (PID)

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WES has become an attractive option for the genetic diagnostics in immunology. We tested the performance of WES in a cohort of 10 patients with PID selected based on the following criteria: three or more possible candidate genes based on the phenotype, or low amount of DNA available, not allowing for stepwise sequencing of candidate genes. The raw data from HiSeq1500 were analysed using an in-house pipeline and from the resulting vcf files the common variants (<1%) and variants predicted as tolerated by both PolyPhen and SIFT were discarded. Genes related to PID or aplastic anemia were chosen from the remaining variants. This approach identified underlying mutations in 5/10 cases: interleukin-7 receptor in infant with severe combined immunodeficiency (SCID); perforin in infant who died due to familial hemophagocytic lymphohistiocytosis; SLX4 and ASXL1 in two children with refractory cytopenia, and TACI in patient with common variable immunodeficiency (CVID). In another infant with SCID a mutation causing cartilage hair hypoplasia was missed by WES, because the RMRP gene was not covered in the sequencing library. The diagnosis was done by clinical geneticist only after stem cell transplant, which was performed at 2.5 months of age. In three patients (2x CVID, 1x neutropenia) possible disease-causing mutations were suggested but not yet verified. In one patient, no mutation explaining his phenotype (neutropenia, B lymphopenia) was identified. In conclusion, in our limited cohort the WES proved as highly useful but not universal diagnostic tool for difficult cases in immunohematology. *Supported by IGA NT14343 and CZ.2.16/3.1.00/24022OPPK.*

PM07.18
Utility of next generation sequencing (NGS) in primary immunodeficiency disorders (PIDs); cases based review

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Introduction: Primary immune deficiency disorders (PIDs) are a group of diseases that the genetic basis of many has been identified. Although the traditionally diagnostics are often sufficient to confirm the suspicion of certain PIDs, molecular tests are required to permit a conclusive diagnosis. Genetic counseling together with clinical evaluation plays a critical role in the appropriate use of these tests, which have great potential to improve treatment outcomes. In this review by our experiences, we outline the algorithm for PIDs and the clinical utility of next generation sequencing (NGS).
Materials and Methods: We have designed a diagnostic algorithm and customized test panels via flow cytometry and then next-generation-sequencing by MiSeqSystem (Illumina). In-silico analysis for novel mutations was carried out using SIFT, Polyphen2 and MutationTaster.
Results: The causative mutation was identified in only 18 out of 38 patients with suspected PIDs and 9 out of 12 patients with chronic granulomatous disease (CGD). More than that, the identified mutations in 10 of 18 were novel mutations. Many of undiagnosed suspected patients (n=20 for PID, n=3 for CGD) whom immunophenotype does not help to identify them may have had as yet uncharacterized mutations in other genes.
Conclusion: In summary, the availability of molecular genetic testing has profound implications for immunologists and patients. The benefits of genetic testing are for diagnosis including the pre-symptomatic and screening, prevention including the prenatal and pre-implantation genetic diagnosis, treatment even including the gene therapy modalities, prognosis and research.
Moreover, the application of NGS has broadened the understanding PIDs and critical in modern disease classification.

PS07.19
Exome sequencing as a first tier test for primary immunodeficiencies

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Primary immunodeficiencies (PIDs) are a heterogeneous group of diseases characterized by an increased susceptibility to infections, because of genetic defects in one or more components of the immune system. At the moment at least 250 genes/loci are known as genetic causes for PIDs. Exome sequencing (ES) allows simultaneous analysis of a large panel of genes in a single test. We have applied this approach as a first tier diagnostic test for PIDs since May 2013. First, we designed an "in silico" immunodeficiency gene panel which contains 270 genes (proven to be involved in PIDs and a few strong candidate genes). Since then we performed clinical diagnostic exome sequencing in >60 cases, all highly suspicious of having a PID. Analysis of variants present in the immunodeficiency panel, allowed us to establish a genetic diagnosis in 10% of the cases. In 15% of the negative cases we detected one pathogenic mutation in a recessive PID gene. It is not known yet whether this has an influence on the patients phenotype or whether a second mutation might be present. In 80% of the negative cases we performed exome wide analysis. In 15% of these we identified highly suspected variants in genes that likely play a role in the patients phenotype. Additional studies are ongoing to provide stronger causal evidence.

PM07.20
Nakajo-Nishimura syndrome: A mexican report with a new PSMB8 mutation

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Nakajo-Nishimura syndrome (NNS) (OMIM 256040) is a very rare hereditary autoinflammatory disorder with onset in infancy with fever, pernio-like rashes and gradually develops into partial lipodystrophy. It is a proteasome-associated auto-inflammatory syndrome caused by autosomal recessive mutations in the PSMB8 gene (proteasome subunit β type 8) mapped on 6p21.3. Mutations in this gene also causes JASL syndrome (Japanese Autoinflammatory Syndrome with Lipodystrophy), JMP (Joint Contractures, Muscle Atrophy, Microcytic Anemia, and Panniculitis-Induced Lipodystrophy Syndrome) and CANDLE (Chronic Atypical Neutrophilic Dermatosis with Lipodystrophy and Elevated Temperature).We report a mexican female with congenital left cleft lip and palate, low birth weight ans short stature. She had longstanding history of recurrent fevers and associated rash, gradaully developed partial lipodystrophy, developmental delay, pernio-like rashes. Abnormal laboratory tests included elevated triglycerides, IgG, IgE; ANAs were negatives. Different homozygous PSMB8mutations had been identified until now. In order to determinate and to confirm the mutation because of a NNS suspected, PSMB8 mutation screening was performed (6 exones included). We detect a distinct mutation not reported previously: the G783A nucleotide substitution. It causes a change from GCC codón to ACC, corresponding to protein Ala88Thr substitution.

PS07.21
Palmoplantar pustular psoriasis is associated to missense variants in CARD14, but not to loss-of-function mutations in IL36RN in Eueopean patients

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Palmoplantar pustular psoriasis (PPP) is a less common manifestation of psoriasis characterized by sterile pustules on the palms and soles. Little is known on its genetic risk factors. In order to find out whether PPP has genetically more similarities with generalized pustular psoriasis (GPP) or psoriasis vulgaris (PsV), we screened 217 German PPP patients as well as 34 Estonian PPP patients for possible mutations in IL36RN and CARD14 and performed an association analysis of the most relevant genetic risk factor for PsV, the PSORS1 risk allele. We identified four heterozygous carriers of IL36RN mutations, but no evidence for association in comparison to genotyped controls and large, publicly available data of control individuals. Also, our studies confirm previous studies showing no evidence for association of PPP to PSORS1. In contrast, three different rare missense variants CARD14,

previously described to influence the activation of NF-κB, were identified in eight patients and significantly associated to PPP in this case control study (p= 3.81E-04). Thus, our investigations suggest that CARD14 variants might be overlapping risk factors for PPP, GPP and PsV, although, due to their rare frequency in PPP patients, their overall contribution to the genetic risk of PPP - as in other psoriatic entities - will remain small.

PM07.22

Decreased Severity of Experimental Autoimmune Arthritis in Peptidylarginine Deiminase Type 4 Knockout Mice

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Introduction
Peptidylarginine deiminase type 4 (PADI4) has been identified as a susceptibility gene for rheumatoid arthritis (RA) by genome-wide association studies. PADI4 is highly expressed in the bone marrow, macrophages, neutrophils, and monocytes. Peptidyl citrulline is an interesting molecule in RA because it is a target antigen for anti-citrullinated peptide antibodies, and only PADs (translated proteins from PADI genes) can provide peptidyl citrulline via the modification of protein substrates. The aim of this study was to evaluate the importance of the PADI4 gene in the progression of RA.
Methods
We generated Padi4 knockout (Padi4−/−) DBA1J mice. The Padi4−/− DBA1J and wild-type mice were immunized with bovine type II collagen (CII) to develop collagen-induced arthritis (CIA). The expression of various inflammatory cytokines and Padi genes in immune cells was detected by the real-time TaqMan assay. Cytokine concentrations in sera were measured by enzyme-linked immunosorbent assays. Localization of the PAD4 and PAD2 proteins was indicated by immunohistochemistry.
Results
We demonstrated that the clinical disease score was significantly decreased in the Padi4−/− mice and Padi4 expression was induced by CII immunization. In the Padi4−/− mice, serum anti-type II collagen (CII) immunoglobulin M (IgM), IgG, and inflammatory cytokine levels were significantly decreased compared with those in the wild-type mice. Padi2 expression was induced in the immune cells of the Padi4−/− mice as a compensation for the defect in Padi4.
Conclusion
Padi4 affected disease severity in the CIA mice and was involved in the enhancement of the collagen-initiated inflammatory responses.

PS07.23

Whole exome sequencing of Cold Medicine-Related Stevens-Johnson Syndrome/Toxic Epidermal Necrolysis (CM-SJS/TEN) with Severe Mucosal Involvement in Japanese population

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Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are acute inflammatory vesiculobullous reactions of the skin and mucous membranes, and these reactions are reported to be caused by inciting drugs, viral infections, or malignant tumor. Although the occurance of SJS/TEN is rare at about 1-6 cases per million, mortality rates are higher than other drug rush (SJS: 3%, TEN: 27%). In addition, Quality-of-life (QOL) of the most survivors is often low, as severe ocular surface complications are often developed as the aftereffect.
Over 1,000 drugs have been reported as the so-called “causal medicine” of SJS/TEN, and susceptibility to SJS/TEN caused by some of them the medicines have been reported to be associated with HLA genes. As examples, HLA-A*31:01 and HLA-B*15:02 have been reported to be associated with carbamazepine-induced severe cutaneous adverse reactions (SCARs). Besides, HLA-B*58:01 have been reported as susceptible allele of Allopurinol related SJS/TEN. Recently, HLA-A and IKZF1 have been reported to be susceptible for Cold Medicine-Related Stevens-Johnson Syndrome/Toxic Epidermal Necrolysis (CM-SJS/TEN) in Asian populations by genome-wide association study (GWAS). However, remaining genetic susceptibility of CM-SJS/TEN including rare variants and structural variants remains to be discovered.
To identify the functional variants for CM-SJS/TEN with severe mucosal involvement, whole-exome sequencing was performed in about 121 Japanese CM-SJS/TEN patients with severe mucosal involvement using next generation sequencer (NGS) - ion Proton (Thermo-Fisher Scientific). Whole-exome sequencing data were then analyzed using CLC Genomics Workbench (CLC bio) software.
Here we will show the provisional results of Whole-exome sequencing for CM-SJS/TEN. This study illustrates novel diagnostic and therapeutic methods for CM-SJS/TEN.

PM07.24

Targeted high throughput sequencing identifies novel disease candidate genes for systemic lupus erythematosus in Swedish patients

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Systemic lupus erythematosus (SLE) is an autoimmune disorder with heterogeneous clinical manifestations. The etiology of SLE is complex, involving interplay between genetic, environmental and hormonal factors. Although many loci have been identified, only a small proportion of heritability of SLE is explained, indicating a demand for different approaches for discovery of disease-associated genes. The aim of this study was to identify novel rare genetic variants relevant for SLE. We performed targeted re-sequencing of 219 genes selected on the basis of their role in immune responses, general autoimmunity, and known associations with human SLE, or SLE-related disease in dogs. The study cohort included 144 Swedish SLE patients and 17 matching controls. The Nimblegen capture array was used for target regions enrichment, which were sequenced on Illumina HiSeq2000. Variants were filtered to be present only in cases, but not in controls or 1000 genomes or dbSNP. For further functional validation we chose three variants located in the genes MEF2D, TCRA and HAPLN3 and overlapping with DNase I hypersensitivity sites, ENCODE histone marks and ChIP-Seq peaks. The regulatory potential of the novel variants was investigated with EMSA and luciferase reporter assay, and indicated that two variants may influence gene expression. Additional genotyping of 742 Swedish controls demonstrated a higher frequency of the variants in SLE patients. A correlation between particular disease manifestations and the novel variants was also observed. Our study successfully combines the detection of novel rare variants, with functional and clinical data to generate hypothesis about specific disease mechanisms.

PS07.25

Identification of the risk HLA variants of Graves’ disease by applying the HLA imputation method to large scale Japanese genome-wide association study data

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[Objectives] Graves’ disease (GD) risk is strongly associated with genetic variations within the major histocompatibility complex (MHC) region. However, its fine genetic architecture has not been elucidated.
[Methods] To fine-map GD risk alleles within the MHC region in Japanese, we newly constructed a population-specific HLA reference panel consisting of the healthy subjects of Japanese ancestry (n = 908). We conducted trans-ethnic comparisons of linkage disequilibrium (LD) and haplotype structures of the human leukocyte antigen (HLA) variants by introducing a novel framework that utilizes an entropy-based LD measurement and a visualization tool capturing high-dimensional variables. Finally, we applied our HLA imputation method to the large-scale Japanese GD genome-wide association study (GWAS) data (n > 9,000).
[Results] The Japanese population demonstrated stronger LD between the HLA genes compared to European or other east Asian populations, highlighted by one population-specific common long-range HLA haplotype spanning through the entire MHC region and HLA genes (frequency > 0.08). Application of the HLA imputation method to the Japanese GD GWAS data identified that amino acid polymorphisms of the multiple class I and class II HLA genes independently contribute to GD risk (HLA-DPβ1 amino acid positions 35 and 9, HLA-A amino acid position 9, HLA-B amino acid positions 45 and 67, and HLA-DRβ1 amino acid position 74; P < 6.0×10^-7), with the strongest impact at HLA-DPB1 (P < 1.0×10^-40).
[Conclusion] Our study illustrates the value of the population-specific HLA reference panel for HLA imputation, with successful fine-mapping of the MHC region risk of GD to multiple HLA amino acid polymorphisms.

PS08.01
16p13.11 microdeletion in a boy presenting with Mowat-Wilson phenotype

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16p13.11 microdeletion is a recently described syndrome characterized by developmental delay, microcephaly, epilepsy, dysmorphic features and behavioral problems. The deleted region contains more than 10 genes including NTAN1 and NDE1. These genes may be relevant to the neuro-cognitive phenotype of this syndrome. Variable phenotypic presentation has been described with partial penetrance. Most cases are de novo. Prevalence in the normal population is 4:10,000 . However 16p13.11 deletions is detected in 60:10,000 patients with a diverse spectrum of epilepsy syndromes.
Mowat Wilson syndrome (MWS) is a rare condition (1:60,000), characterized by a distinctive phenotype, mild to severe develeopmental delay, seizures, short stature, microcephaly, Hirschsprung disease or constipation, genitourinary defects, heart defect, and eye anomalies. Almost all cases are associated with ZEB2 mutations, located on chromosome 2q22.3.
We describe a 12y old boy with intellectual disability, behavioral problems, history of infantile spasms since the age of 3m, constipation, hypospadias, dysmorphic features and microcephaly. Parents are healthy and unrelated. He has a non-identical twin brother who has ADD but is otherwise healthy and has normal intelligence. MWS has been considered in this boy. However, routine workup included CMA testing diagnosed a 16p13.11 microdeletion. Clinical reevaluation of the patient in view of the CMA results revealed the overlapping between both syndromes.
We would like to suggest that 16p13.11 microdeletion may be a phenocopy of MWS. CMA in our report showed once again its importance in resolving cases with intellectual disability and suggested the existence of gene(s) in the deleted interval that may be responsible for a phenocopy of MWS.

PM08.02
De novo 19p13.2 microdeletion encompassing the part of NFIX gene in a girl with Sotos-like phenotype

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19p13.2 microdeletion is a rare genomic disorder. To date, less than ten cases was described so far in the literature. This chromosomal syndrome manifests in developmental delay/intellectual impairment, postnatal overgrowth, macrocephaly, craniofacial dysmorphy, slender habitus, advanced bone age, and scoliosis. Hypotonia, unusual behavior with autistic traits, ophthalmologic, gastrointestinal, and hand/foot abnormalities are also frequent. Very similar phenotype is observed in the Sotos syndrome 2 (MIM #614753) that is caused by heterozygous mutations/deletions of the NFIX gene located on chromosome 19p13.2.
Here, we report on the case of a 7-year-old girl referred to genetic counseling because of psychomotor retardation, speech delay and dysmorphic features (macrocephaly, long/narrow and triangular face, high forehead, frontal bossing, downslanting palpebral fissures, dysplastic low-set ears, small nose, flat philtrum, micrognathia). At the examination, behavioral problems (anxiety, tantrums, autoaggression), seizures, and poor coordination were noted. Skeletal anomalies (advanced bone age, joint laxity, slender hands with long fingers, abnormal setting of the lower limbs), strabismus, hyperopia as well as constipations and abdominal pain were also diagnosed.
Whole-genome oligonucleotide microarray analysis revealed a de novo 158.18 kb (the smallest so far) deletion of 19p13.2 region (chr19:13,020,206-13,178,390; hg19) encompassing seven genes; three of them (NFIX, DAND5 and CALR) could be the candidate genes for the genotype-phenotype relationship.
This study contributes additional information for the newly identified 19p13.2 microdeletion syndrome and clarifies the clinical roles of genes in the involved region. Our results also confirm that haploinsufficiency of NFIX leads to Sotos-like phenotype.
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PS08.03
Phenotype and natural history of 1p36 deletion syndrome in five adult patients

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1p36 deletion syndrome is one the most common subtelomeric deletion syndromes seen in humans. Uniform features of the syndrome include intellectual disability, characteristic dysmorphic facial features, cardiac and craniofacial anomalies. This syndrome has been well described in paediatric patients. However, in adult patients data on the natural history is still limited.
We describe five patients with a microdeletion 1p36, that were diagnosed during adulthood, to illustrate the variability of the clinical course. The most severe case was a male patient that was diagnosed with 1p36 deletion syndrome at the age of 18 years. He had a profound intellectual disability, seizures, challenging behaviour and bilateral congenital cataract. A few weeks after diagnosis he developed stroke caused by a newly discovered dilated cardiomyopathy, which is a common feature of 1p36 deletion syndrome. Unfortunately, 6 months later he died of heart failure that was exacerbated by a respiratory infection. This case illustrates the importance of early genetic diagnosis and syndrome specific follow-up in adult patients, to provide better care and early intervention in this challenging group of patients.

PM08.04
2p15-p16.1 microdeletion syndrome: further refining the critical region

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The 2p15-p16.1 microdeletion syndrome is a rare disorder characterized by developmental delay, intellectual disability, growth retardation, microcephaly, abnormal muscle tone, optic nerve dysplasia and a distinctive pattern of craniofacial features. Reported deletions' sizes range from 5.7 Mb, containing numerous genes, to 0.2 Mb, including a single gene, BCL11A.
We report a 4-year-old girl with delayed motor and language development, microcephaly, asymmetry in carpal bone maturation, facial hypotonia and peculiar craniofacial features. Array-CGH revealed a de novo deletion of 350 Kb in the locus 2p16.1, which only included a microRNA MIR4432 and part of the BCL11A gene, and a duplication of 123 Kb in 22q13.1, inherited from the proband's healthy mother. To our knowledge, this is the second smallest deletion associated with the 2p15-p16.1 microdeletion syndrome reported to date. Compared to the individual with the 0.2 Mb deletion, who presented with mild intellectual disability, abnormal muscle tone and expressive language delay, our proband also shows microcephaly and facial features evocative of the condition, while growth retardation and other features described in previous cases are absent.
In conclusion, the present case provides further evidence on the correlation of heterozygous 2p15-p16.1 deletions with specific clinical features. Furthermore, the small size of the current deletion might help to clarify the role of single genes in this complex phenotype.

PS08.05
2p25 deletion: involvement of SNTG2 in autism or behavioral troubles, and parental imprinting.

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Deletions of the terminal region of the short arm of chromosome 2 have been reported in the literature in less than 20 patients. These patients share common clinical features including early-onset obesity, intellectual disability and behavioral troubles or autism. A minimal critical region of 1.97 Mb has been estimated, encompassing seven genes (*SH3HYL1*, *ACP1*, *TMEM18*, *SNTG2*, *TPO*, *PXDN*, and *MYT1L*). Many recent studies pointed out *MYT1L* gene as the main candidate for ID and obesity. We present three new patients carrying 2p25 deletions: an autist female child with developmental delay and her depressive father, and a third unrelated patient who has psychomotor retardation and behavioral troubles. Moreover, our patients do not have an overweight phenotype, highlighting indeed the role of *MYT1L* in obesity, as none of the deletions encompasses *MYT1L*. The only common disrupted gene is *SNTG2*, thus redefining the smallest region of overlap for autism. *SNTG2* is expressed in brain. Its product interacts with neuroligins NLGN3, NLGN4X, and NLGN4Y, which are autism-related isoforms. Finally, the parental origin of the deletions seems to be of interest, as all inherited deletions described today appear to be inherited from the paternal side. We will discuss the possible imprinting of the 2p25 region and its role in autism.

PM08.06

A milder Angelman syndrome phenotype due to a novel molecular mechanism: a maternally inherited 30kb deletion of 15q11.2 including the main gene promoter and non-coding exon1 of UBE3A

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We present a novel molecular cause of Angelman syndrome (AS) in a female patient with microcephaly, dysmorphism, global developmental and speech delay and intellectual disability. Her phenotype resembles a milder form of AS with no seizures and 20 words of speech at the age of 5 years. EEG investigation of the patient showed typical AS findings.
Suspecting a clinical diagnosis of AS, molecular analysis was undertaken using methylation specific PCR (MS-PCR) and revealed normal methylation at the SNRPN DMR ruling out UPD, deletion or an imprinting defect associated with the majority of AS. Sequencing of the coding exons of UBE3A revealed no pathogenic mutations. Microarray CGH identified a maternally inherited 30kb deletion at 15q11.2 involving exon 1 of UBE3A. This deletion was de-novo in the patient's mother and linkage analysis revealed the origin of the deletion in the grand-paternal allele, explaining the normal phenotype in the patient's mother due to genomic imprinting.
Although intragenic deletions of the maternal copy of UBE3A are a recognised cause of AS, to date there have been no reported deletions of exon 1. Exon 1 is non protein coding and therefore not routinely sequenced. The intragenic deletion in our patient also includes the main promoter of UBE3A adjacent to exon 1 and the milder phenotype in our patient may be explained by secondary promoters downstream producing transcripts.
We present the detailed clinical phenotype of our patient, discuss the initial difficulties of interpreting the clinical significance of this novel finding and present the subsequent RNA/expression analysis.

PS08.07

Mosaicism of truncanjting mutation in CASK is crucial for male patients' survival

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The CASK gene maps to Xp11.4 and is related to synaptic functions. Aberrations of this gene are associated with microcephaly with pontine and cerebellar hypoplasia (MICPIH), developmental delay, and nystagmus in females. We report a male patient with mosaicism of CASK mutation.
He was born to nonconsanguineous parents at 39 weeks. The parents were healthy and their family history was unremarkable. His birth weight was 2334g (-1.4SD), length 45cm (-1.5SD), and the head circumference 30.0cm (-2.2SD). He had ventricular septal defect, and had mitral valve regurgitation at 5 months old. He also had nystagmus. At 3 years and 6 months old, his weight was 9075g (-3.2SD), length 82.0cm (-4.0SD), head circumference 39.0cm (-7.2SD). He had severe intellectual disability. At 4 years old, he doesn't speak, nor does he keep sitting position. Chromosome studies showed a normal 46,XY karyotype. We performed cytogenetic microarray, which revealed no significant copy number variation. Next we performed targeted high-throughput sequencing of 4813 genes in which mutations had been described in patients with Mendelian genetic disorders. We confirmed a de novo mosaic mutation (c.725G>A:p.W242X) of CASK. The ratio of mutation was 93% in peripheral blood lymphocytes.
CASK knockdown mice are smaller than wild type mice but viable, whereas CASK knockout mice die at first day of their lives. For male patients, truncating mutation in CASK has been regarded as lethal. Next-generation sequencing confirmed a mosaicism masked by a high proportion of mutant cells. These results suggest that mosaicism seemed crucial for male patients' survival.

PM08.08

Homozygous deletion of CHL1 resulting in severe intellectual disability

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Hemizygous deletion of the CHL1 gene at 3p26.3 is a very rare finding, previously reported in 4 families. Cognitive impairment, in particular language difficulties, is a consistent phenotypic feature. However, due to the small number of cases reported to date, the clinical significance of deletions including CHL1 remains uncertain. Homozygous deletions of the region have never been reported.
Here we describe an eight year old boy, born to consanguinous parents, who has marked learning difficulties, absence of speech, stiffness in his lower legs and persistent drooling. Array CGH studies carried out using the BlueGnome CytoChip Oligo ISCA 8x60k (v2.0) array showed a deletion of the sequences detected by the oligonucleotide probes located from 93,979bp to 290,767bp. This equates to a 197kb interstitial deletion of the short arm of chromosome 3 at p26.3. This resulted in a partial deletion of the CHL1 gene. Real-time quantitative PCR (qPCR) studies with targets within introns 1 and 2 of the CHL1 gene, confirmed this deletion and showed that it was homozygous. Further qPCR studies in the parents of this proband showed they both had a hemizygous deletion of this region. The proband has therefore inherited one abnormal chromosome 3 from each parent who are both clinically unaffected.
This is the first case reported of a patient presenting with an abnormal phenotype with a homozygous deletion including part of the CHL1 gene at 3p26.3.

PS08.09

Copy number variant at Xp22.31: Clinical and genetic evaluation of 8 patients

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Introduction:
Xp22.31 submicroscopic duplication has been reported as either a possible cause of intellectual disability (ID) and/or developmental delay or as a benign variant.
Methods:
Comparative genomic hybridization array (aCGH) (Agilent 60K) was used to analyse 511 patients affected by ID and/or dysmorphic features/congenital defects.
Results:
We detected potentially pathogenic copy number variants (CNV) in 94 out of 511 patients analysed (18%). A submicroscopic duplication in the Xp22.31 region was identified in 8 patients (5♀:3♂) ranging in size from 257 Kb to 1.7 Mb. In all individuals studied (four out of eight) the Xp22.31 duplication was inherited from the mother. The HDHD1, STS, VCX and PNPLA4 genes are included in four cases with a 1.6Mb duplication, who show autism spectrum disorder (ASD) and no dysmorphic features. Interestingly, two cases with a size below 580Kb, where only HDHD1 and STS genes are duplicated, show a more severe phenotype with multiple malformations. The largest duplication detected (from VCX3A to VCX2) is associated with language delay. The patient also carries a deletion at 6p23 of 1.3 Mb as a result of an unbalanced t(6;9). Finally, a duplication of 565Kb involving VCX and PNPLA4 was detected in a boy with psychomotor delay.
Conclusion:
We have detected an association of the Xp22.31 duplication with ID and ASD in both genders. According to the literature, there is a high variability in the Xp22.31 duplication associated phenotype, which may be due to incomplete penetrance, the different genes involved, a position effect and X-inactivation. Increased CNV on the X chromosome can contribute to deregulation of normal cognitive development.

PM08.10

Partial ARID1B gene deletion as a cause of Coffin-Siris syndrome in two (maybe three) siblings

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Coffin-Siris syndrome has been described as mental retardation associated with coarse facial features, hypertrichosis, and hypoplastic or absent fifth fingernails or toenails and other variable clinical manifestations. We report the case of a non consanguineous portuguese couple. Their first pregnancy of a female fetus was interrupted at 23 weeks because of corpus callosum agenesis and cardiac defect. No genetic analysis or autopsy was requested at this time. Their second pregnancy of a female fetus was also characterized by corpus callosum agenesis, and unique umbilical artery. At 2 years of age, she is affected by hydrocephalus, laryngomalacia, diaphragmatic hernia,coarse facial features, hypoplastic fifth toenails. No genetic analysis was requested before we saw her. Their third pregancy was characterized by a male fetus with left hydronephrosis, unilateral club foot, and corpus callosum poorly visualized. Microarrays CGH analysis was requested for the third fetus, using a 60K Agilent whole genome array. It revealed a 230 kb intragenic deletion of ARID1B gene (exons 6-10) at position 6q25.3. This discovery was clearly

suggestive for the phenotype of the living daughter and the analysis found the same deletion confirming a clinical suspicion of Coffin-Siris syndrome. Medical interruption of the third pregnancy was asked by the parents at 23 weeks, but no autopsy was requested. Parental CGH microarrays testing did not reveal any copy number abnormality at this 6q25.3 region. However, familial history with two siblings affected (possibly three but no genetic material was conserved for the first pregancy to verify this hypothesis) strongly suggest that one of the parents is a carrier of a germinal mosaicism.

PS08.11

Scavenging mRNA for Cognitive Function

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mRNA decay is an essential and active process that allows cells to continuously adapt gene expression to internal and environmental cues. There are two mRNA degradation pathways: 3' to 5' and 5' to 3'. The DCPS protein is the scavenger mRNA decapping enzyme which functions in the last step of the 3´ end mRNA decay pathway. We have identified a DCPS pathogenic mutation in a large family with three affected individuals presenting with a novel recessive syndrome consisting of craniofacial anomalies, intellectual disability and neuromuscular defects. Using patient's primary cells, we show that this homozygous splice mutation results in a DCPS loss-of-function allele. Diagnostic biochemical analyses using various m7G cap derivatives as substrates reveal no DCPS enzymatic activity in patient's cells. Our results implicate DCPS and more generally RNA catabolism, as a critical cellular process for neurological development, normal cognition and organismal homeostasis in humans.

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PM08.12

Arginine supplementation in De Barsy syndrome due to ALDH18A1 mutations

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De Barsy syndrome is a rare autosomal recessive disease characterized by intrauterine and postnatal growth retardation, progeroid appearance, corneal clouding, joints hypermobility, osteopenia, athetoid movements and mental retardation. Dysmorphic aspect of the face and the skin includes microcephaly, frontal bossing, large dysplastic ears, thin lips, cutis laxa, wrinkled atrophic skin and reduced subcutaneous fat. To our knowledge, only two cases of De Barsy syndrome associated with blood vessels tortuosity have been described.

De Barsy syndrome is genetically heterogeneous since 2 genes have been identified: ALDH18A1 encoding delta1-pyrroline-5-carboxylate synthase (P5CS) and the gene encoding pyrroline-5-carboxylate reductase 1(PYCR1). Both genes are involved in proline biosynthesis pathway but pathological mechanisms leading to the syndrome are still misunderstood. Some patients carrying homozygote ALDH18A1 mutations have low level of proline, arginine, citrulline and ornithine.

We report the case of a boy displaying all the features of De Barsy syndrome including specific profile of plasmatic amino acid chromatography. He had also arterial tortuosity (which has been already reported twice in ALDH18A1 mutated patients) and his CT scan showed calcifications which has never been described yet in De Barsy syndrome. This patient carried compound heterozygote ALDH18A1 mutations.

According to the role of P5CS in the proline biosynthesis and as it was already done for another patient of the literature, supplementation with arginine was tried during one year but no improvement was observed on the psychomotor development. This failure suggest that disturbance of proline biosynthesis in P5CS deficiency would not be responsible of the neurological phenotype.

PS08.13

Diagnostic odyssey in severe neurodevelopmental disorders: The utility of clinical whole-exome sequencing

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Objective: The current standard of care for diagnosis of severe intellectual disability (ID) and epileptic encephalopathy (EE) results in a diagnostic yield of ~50%. Affected individuals nonetheless undergo multiple clinical evaluations and low-yield laboratory tests often referred to as a "diagnostic odyssey". This study was aimed at assessing the utility of clinical whole-exome sequencing (WES) in individuals with undiagnosed and severe forms of ID and EE, and the feasibility of its implementation in routine practice by a small regional genetic center.

Methods: We performed WES in a cohort of 43 unrelated individuals with severe forms of ID and/or EE. All individuals had undergone multiple clinical evaluations and diagnostic tests over the years, with no definitive diagnosis. Sequencing data analysis and interpretation were carried out at the Dijon University Hospital Molecular Genetics Laboratory.

Results: The molecular diagnosis rate of WES reached 32,5% (14 of 43 individuals), with pathogenic or likely pathogenic variants associated with six autosomal dominant (ARID1B, CTNNB1, DYRK1A, SHANK3, NAA10 and TBR1), five autosomal recessive (ADCK3, GFER, SCN10A, TAF2 and SLC13A5 in two families) and two X-linked (CUL4B, and SLC16A2) conditions. Genetic diagnosis had implications for a personalised clinical management in four families, including prenatal diagnosis test in one family.

Interpretation: Our data emphasize the clinical utility and feasibility of WES in individuals with undiagnosed forms of ID and EE, highlight the necessity of close collaborations between ordering physicians, molecular geneticists, bioinformaticians and researchers for enhanced data interpretation, and support the use of WES as a first-line test for individuals with severe neurodevelopmental disorders.

PM08.14

DYRK1A haploinsufficiency, a novel frequent cause of microcephaly and developmental delay?

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DYRK1A has been the most extensively studied chromosome 21 gene during the last decade due to the correlation with Down syndrome neuropathologies. It is known to be expressed in adult human brain with the highest level of expression in the cerebellum. It is closely linked with brain growth in a gene dosage dependent manner. DYRK1A haploinsufficiency induces a reduced brain size in mice whereas a DYRK1A gain-of-function model would exhibit increased brain volume. DYRK1A has been implicated in tau phosphorylation and is becoming an attractive drug target since its overexpression may induce Down Syndrome-like neurobiological alterations.

Deletions or mutations involving DYRK1A have been reported in association with intellectual disability (ID) and microcephaly. We have identified 3 *de novo* deletions at 21q22.12 including DYRK1A and 8 DYRK1A *de novo* mutations in 11 unrelated patients through exome sequencing via the DDD project and other collaborations. All patients presented with microcephaly and ID. Ten patients presented with growth retardation, epilepsy and behaviour issues. Additional features such as cleft palate, eye, brain and heart malformations were identified.

We will review our patients' findings and compare them with those previously reported to further delineate the phenotypic spectrum of patients with DYRK1A abnormalities. We will also review public expression data set on fetal and adult human brain to understand the underlying molecular mechanisms for this emerging syndrome.

DYRK1A should be added to the list of genes responsible for syndromic ID

comprising microcephaly and epilepsy, such as ARID1B, SCN2A, ANKRD11, SATB2 and SYNGAP1.

PS08.15

Myosin-18B and mathematical ability in independent cohorts: lack of replication in independent cohorts

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Dyscalculia (or mathematical ability) is a condition where mathematical ability is severely impaired. Twin studies suggest that it is partly caused by a genetic component, which is yet to be understood at the molecular level. Recently, a coding variant (rs133885) in the Myosin-18B gene was shown to be associated with mathematical abilities with a specific effect among children with dyslexia. This association represents one of the most significant genetic associations reported to date for mathematical abilities. However, this association has not been replicated before. We conducted a replication analysis in different cohorts characterised with maths-related measures. The study was conducted primarily using the Avon Longitudinal Study of Parents and Children (ALSPAC) (N=3819), which was adequately powered for this analysis. We tested additional cohorts including the York Cohort (N=291), the Specific Language Impairment Consortium (SLIC) (N=367) and the Raine Cohort (N=667). Cohorts were stratified for a definition of dyslexia where possible. We did not observe any associations between rs133885 in Myosin-18B and mathematical abilities among individuals with dyslexia or in the general population. Our results then suggest that the Myosin-18B variant is unlikely to be a main factor contributing to mathematical abilities.

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PM08.16

MTRNR2L12: A candidate blood marker of early Alzheimer's disease-like dementia

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Histological changes constantly reported in autopsied brain tissue of adult patients with Down syndrome are identical with those observed in Alzheimer's disease. Interestingly, many adults with Down syndrome never present with cognitive deterioration whereas in others, Alzheimer's disease-like dementia develops already in the fifth decade of life.

The aim of the study was to identify variations of genome expression in blood that could serve as markers of early dementia in adults with Down syndrome.

Methods:

Cognitive assessment was performed in a cohort of 48 adults with Down syndrome with subsequent microarray-based analysis of whole genome expression in leukocytes. The participants of the study were stratified with regard to their age and cognitive status to allow for comparison of group-specific expression profiles.

Results:

Analysis of microarray data revealed highly significant differences between groups of younger patients with severe cognitive disability and of older patients without dementia with regard to expression of *MTRNR2L12* gene, which is known to be associated with Alzheimer's disease. Subsequent comparison of another subgroup of younger patients with documented cognitive deterioration and the above group of older patients without dementia gave very similar results.

Conclusion:

The findings of the study suggest a protective role of MTRNR2L12 in development of early Alzheimer's disease-like dementia in adults with Down syndrome. Further studies should be performed to evaluate potential usefulness of this marker in patients with Alzheimer's disease.

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PS08.17

Comprehensive genetic and metabolic investigation of undiagnosed early onset epileptic encephalopathies

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Early-onset epileptic encephalopathies (EOEEs) represent a heterogeneous group of neurological disorders, often characterized by therapy-resistant seizures, electroencephalographic abnormalities as well as motor and cognitive deficits or decline. Despite the poor prognosis, early diagnosis in affected children may contribute to improve disease management and enables genetic counseling. Due to excessive heterogeneity in EOEEs, clarification of the etiology is difficult. We therefore studied the usefulness of comprehensive genetic and metabolomic testing in 63 consecutive patients with EOEE followed in the pediatric neurology division. We performed high-resolution chromosomal microarray analysis (CMA) using the Affymetrix Cytoscan HD array and high coverage (average more than 290fold) whole exome trio sequencing (WES) using the Illumina HiSeq 2500 platform. 6.3% of patients showed clearly pathogenic CNVs. In 5 (26.3%) out of 19 trios analyzed so far by WES, deleterious mutations in disease causing genes were identified; 2 (40%) of them represent autosomal dominant mutations, 2 (40%) were inherited in an autosomal recessive pattern and 1 (20%) was an X-linked condition. Furthermore, in another 5 (26.3%) trios we found deleterious variants in genes previously reported in association with epilepsy and/or ID, but not described in EOEEs. Metabolome analysis in plasma was so far completed in 36 patients and revealed abnormal profiles in 5 patients. In one case a novel plasma biomarker could be identified and matched with the mutated gene supporting the functional relevance of the respective mutation. Therefore, CMA and WES is a powerful diagnostic tool in EOES revealing a diagnosis in at least 33% of patients.

PM08.18

Genetic diagnosis in early infantile epileptic encephalopathy and severe neurodevelopmental delay using a gene panel: Our experience and results so far.

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The early infantile epileptic encephalopathy (EIEE) syndromes are a heterogeneous group of conditions characterised by intractable seizures and developmental delay or regression. We describe the successful implementation of a gene panel into diagnostic service, our experience so far and the results from 400 patients.

Two different custom enrichments (SureSelect, Agilent and TSCA, Illumina) were used to target 45 genes associated with EIEE and developmental delay followed by sequencing on the Illumina MiSeq. The custom SureSelect panel performed significantly better than TSCA and is now our chosen platform for this panel which has now been expanded to 66 genes.

Mutations in 21 different genes were identified in 67 patients giving a detection rate of 17%. The most frequently mutated genes were SCN2A (11 patients), CDKL5 and KCNQ2 (6 patients each). We found mutations in a number of genes in patients with electroclinical phenotypes not typical for the gene. Given genetic heterogeneity and phenotypic pleiotropy in EIEE, the panel is proving a useful and popular diagnostic tool for Neurologists and Clinical Geneticists, thereby allowing better disease prognostication and genetic counselling for these families, as well as reducing the number and cost of conventional diagnostic tests.

PS08.19

Recurrent CNVs within 17q12 region found in Bulgarian patients with complex epileptic syndromes

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Copy number variations (CNVs) often result from non-allelic homologous recombination (NAHR) during meiosis. They are frequent cause of intellectual disability (ID) and epilepsy. In the present study we performed comparative genomic hybridization (aCGH) using Agilent Microarray Kit, 4x180K in a sample of 71 Bulgarian patients with epilepsy and ID.
In 11 patients recurrent microaberrations within 17q12 region were found, approximately 1 Mb distal from the breakpoints of the classical 17q12 deletion/duplication syndromes. Four of them carried microduplications and seven - microdeletions. All of the examined patients show overlapping phenotype, involving generalized tonic-clonic, myoclonic and absence seizures, ID, behavior problems, speech impairment. Some additional features are ataxia, hypotonia, facial dysmorphisms and memory problems. All of the CNVs vary in size between 95 and 359 Kb, but the minimum shared region includes CACNB1 gene, encoding beta subunit of voltage- dependent Ca2+ channel. This gene is suggested as a strong candidate, responsible for epilepsy in mice. Moreover, mutations of a paralog of CACNB1- CACNB4 are associated with some types of epileptic conditions. Three of the deletions encompass part of FBXL20 gene, implicated in brain functions by modulating neuronal synaptic plasticity. This gene is ubiquitously expressed in brain and loss of function mutations in mice cause histopathological and behavior abnormalities. Therefore, disruptions of FBXL20 gene might contribute to the complex epileptic phenotype seen in our patients. Further investigations are needed to elucidate the effect of the microaberrations and to clarify the phenotype-genotype correlations.
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PM08.20

Mendelian disorders through exome sequencing

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BACKGROUND
For the identification of molecular defects in patients with suspected genetic disorders, whole exome sequencing (WES) has now entered in medical practice as a diagnostic approach and trios analysis as the most effective strategy to identify *de novo* causal variants.
METHODS
We performed exome sequencing using the Ion AmpliSeq™ Exome technology (Life Technologies) with Ion ProtonTM. Sequencing reads were analysed using Torrent Suite software. Trio annotated variants using ION Reporter were prioritized with an in-house analytical pipeline to identify causative genetic variants.
RESULTS
We present data on the first 50 probands for whom referring physicians ordered whole-exome sequencing trio analysis. Patients were mainly children with neurologic disorders including developmental and/or speech delay, autism, and intellectual disability. We identified 5 pathogenic and 14 probably pathogenic mutated alleles that were highly likely to be causative in 18 of the 50 patients, achieving a 36% molecular diagnostic rate. Among the 18 patients, 1 had X-linked dominant, 2 X-linked recessive, 1 autosomal recessive, and 14 autosomal dominant diseases. The X-linked dominant variant and all of the autosomal dominant mutant alleles occurred "*de novo*". Additionally, variants of uncertain significance were identified in 21 patients. These could be novel genes, however further studies are needed to define its role as causative events.
CONCLUSIONS
We have identified the underlying genetic defect in 36% of consecutive patients referred for evaluation of a possible genetic condition using WES. These data allow us to conclude that trio analysis is a powerful and effective diagnostic method for mendelian disorders.

PS08.21

A rare instability event in the FMR1 locus: clinical, molecular and genetic counselling implications

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Fragile X syndrome (FXS) is caused by an expansion of a CGG repeat in the 5'UTR region of the FMR1 gene to over 200 triplets. In typical FXS cases, silencing of the FMR1 gene due to methylation of its promoter precludes protein expression. Loss of the FMR1 protein leads to the physical, neurocognitive and behavioural FXS features. Somatic mosaics in the FMR1 locus are uncommon and can be due either to the presence of alleles with various CGG repeat sizes or epigenetic differences in the extent of methylation. Mosaicism for more than two alleles is a particularly rare finding, although it has been previously described. These phenomena hamper prediction of the disease prognosis.
Herein, we report two independent male cases with a phenotype compatible with mosaic FXS who show atypical mosaic patterns for CGG repeat number, one a mosaic for a full mutation/normal allele and the other for a full mutation/premutation/normal allele. Their mothers were carriers of the premutation. Southern blot analysis, still considered the gold standard for molecular diagnosis of FXS, enabled the characterization of different size mosaics in both cases.
The mechanisms of trinucleotide repeat instability in the FMR1 locus are still not fully understood, although several have been proposed. Likewise, it is still not clear when the repeat expansion occurs (pre-zygotic vs. post-zygotic models). We discuss the possible mechanisms of repeat instability in our cases and present arguments in favour and against both repeat expansion models. Implications for clinical management, molecular diagnosis and genetic counselling are discussed.

PM08.22

Rapid and cost-effective first-tier tool for screening *Fragile X Mental Retardation 1* expansions using triplet-primed PCR and melt curve analysis

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Introduction: CGG-triplet repeat expansions in the 5'-untranslated region of the X-linked *Fragile X Mental Retardation 1* (*FMR1*) gene are associated with three *FMR1* related-conditions, including fragile X-associated tremor/ataxia syndrome, fragile X-associated primary ovarian insufficiency and fragile X syndrome. The high-cost factor and/or the labor-intensive nature of the currently available molecular diagnostic approaches prohibit their application as a first-tier screening test for *FMR1* mutation in large-scale population-based screening programs. We tested the utility of a simplified first-line screening strategy that relies on melt peak temperature (T_m) analysis of direct triplet-primed PCR amplicons (dTP-PCR MCA). Correlation between T_m and CGG-repeat size based on capillary electrophoresis (CE) of dTP-PCR amplicons was also evaluated.
Materials and Methods: The assays were initially optimized on 29 *FMR1* reference DNA samples, followed by a blinded validation on 107 previously characterized patient DNA samples.
Results: Samples carrying an expanded allele generated dTP-PCR melt profiles with pronounced rightward shift to higher T_m range. All normal and expansion carriers were accurately classified by dTP-PCR MCA, and the *FMR1* genotypic classification of the clinical samples was completely concordant with the previously determined genotypes as well as the dTP-PCR CE results. In addition, we also tested the ability of the assays in detecting low-level mosaicism for *FMR1* expansion using artificial DNA mixtures.
Conclusions: When used in a large-scale screening setting, this simple and cost-effective MCA-based first-line screening tool could rapidly screen out the large majority of unaffected individuals, thus minimizing the number of samples that need to be analyzed by Southern analysis.
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PS08.23

A novel HCN1 mutation not associated with epileptic encephalopathy

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The hyperpolarization-activated cyclic nucleotide-gated channel (HCN1-4) family has important roles in the control of heart rate and neuronal excitability. HCN channels selectively conduct K+/Na+ inward current after hyperpolarized potential. In neurons, HCN channels participate in a variety of functions among which excitabily, dendritic integration and plasticity. Animal models have indicated that dysregulation of these channels is associated with several forms of epilepsy including febrile seizures, absence and temporal lobe epilepsy as well as other neurological disorders. Recently HCN1 point mutations have been found in individuals with early onset epileptic encephalopathy with features resembling Dravet syndrome but with different progression over time. All of them developed intellectual disability and autistic traits.
Here we report the case of a 9 year old girl with a Rett like phenotype, characterized by intellectual disability, hand stereotypies, absent speech, negative brain MRI and EEG data and no history of epilepsy. By NGS approach, with the use of a targeted intellectual disability/epilepsy gene panel, we found the girl to carry a novel de novo heterozygous missense mutation in exon 4 of the HCN1 gene. This mutation is located in the glycine-tyrosine-glycine (GYG) motif, which constitutes the ion selectivity filter typical of K+ permeable channels. This motif is highly conserved structurally and functionally during evolution.
This is the first report of a HCN1 mutation involved in a neurodevelopmental phenotype without epilepsy.

PM08.24

A novel HDAC8 mutation in a two-year-old girl with a CdLS-overlapping features but unrecognizable facial gestalt

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The HDAC8 gene encodes a regulatory factor of the cohesin complex. While several mutations in HDAC8 have been identified in patients with Corneliade-Lange-syndrome (CdLS) one single mutation identified in a large family with Wilsson-Turner-syndrome (WTS).
We have performed gene-panel-sequencing analysis on a two-year-old girl with unclear diagnosis and found a novel de-novo HDAC8 mutation. The identified mutation (c.910+1G>A) affects the conserved splice-donor site of the HDAC8 gene and is predicted to results in an aberrantly spliced mRNA that might be degraded by nonsense-mediated mRNA decay or encode a truncated HDAC8 protein.
The patient was born to healthy non-consanguineous parents, aged 38 mother and 42 father. The pregnancy and delivery were uneventful, born at 38 w.g., birth weight 2700 g, birth length 47 cm. The girl presented with a growth delay (length at -4SD, weight -3SD, head circumference -3SD), delayed fontanel closure, and delayed teeth eruption. She showed delayed psychomotor development, sitting steadily at the age of 15 months, did not walk and had no speech by the age of two years. The following dysmorphic features and anomalies were noticed: round face, low forehead, hypertelorism, deep-set eyes, epicanthus, concomitant convergent strabismus, entropion, astigmatism, thick eyebrows, synophrys, long eyelashes, hypoplastic supra-orbital ridges, flat zygomatic region, large nose with bread prominent base, prominent columella, short philtrum, thin lips, medial cleft palate, micrognathia, posteriorly rotated dysplastic ears, short neck, rocker-bottom feet, and impaired conductive hearing. Brain MRI showed hypoplastic vermis and wide ventricles. No inner organ anomalies.
The phenotypical spectrum of HDAC8 mutations is very broad and may not necessarily be recognized as CdLS.

PS08.25

Chromosome 1q21.1 recurrent imbalances in patients with developmental delay revealed by Array-CGH

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Chromosome region 1q21.1 is structurally very complex formed by several segmental duplications that make it prone to non-allelic homologous recombination (NAHR). It can be divided into a proximal region containing 16 genes and a distal region containing 13 genes. Array-Comparative Genomic Hybridization (array-CGH) has led to the identification of new syndromes including microdeletion 1q21.1 and 1q21.1 microduplication. Clinical features of patients include intellectual disability (ID) and dysmorphic features. Clinical variability and lack of distinct facial dysmorphisms have been reported in individuals affected by these imbalances, making genetic counseling very challenging.
Oligonucleotide array-CGH analysis using an Agilent 180K platform was performed in 1200 patients with ID, autism spectrum disorders (ASD) and congenital anomalies. Fluorescence in situ hybridization (FISH) analysis usingc SureFISH probes was performed in patients for validation and in parents to determine the origin of 1q21.1 rearrangements.
We identified 8 probands with imbalances involving the distal 1q21.1 region (6 deletions and 2 duplications) and 1 proband with a duplication involving the proximal region. Inheritance was unknown in 5 patients, 2 microdeletions had arisen de novo and 2 microdeletions was inherited from an unaffected mother. All 9 patients with 1q21.1 imbalances revealed mental retardation, microcephaly/macrocephaly and dysmorphic features.
Array-CGH is a useful tool for 1q21.1 rearrangements screening. Duplications were less frequent in our cohort, consistent with recent studies for NAHR. Patients with 1q21.1 imbalances have a considerable phenotypic diversity that could be associated with incomplete penetrance and variable expressivity. Family studies and further clinical data are essential to improve genetic counselling.

PM08.26

PTBP1 Gene Synonymous Mutation In Intellectual Disability

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Objectives: A patient with intellectual disability, developmental delay and basal ganglia abnormal signal on MRI was evaluated for disease gene identification. We aimed to identify the gene responsible for this patient's phenotype.
Methods: The patient is Jordanian Arab from a consanguineous marriage. Samples from her parents and two unaffected sisters were also collected and DNA extracted. Homozygosity mapping was analyzed using Illumina chip microarray. Candidate gene was sequenced using Sanger sequencing. Bioinformatic analysis was done utilizing web-based tools. **Results:** Different homozygosity intervals were characterized. *PTBP1* (poylpyrimidine tract-binding protein 1) gene was identified as a candidate gene within a homozygous interval. *PTBP1* relative expression level contributes to establishing neural-specific alternative splicing patterns and is a key player in alternative splicing of many genes associated to lineage-specific cell differentiation. Sequencing *PTBP1* identified a homozygous silent mutation in exon 7 (c.708C>T, pH236H) that co-segregated within the family.
This mutation was not reported in any database before nor was it detected in 400 control chromosomes from the same population. Bioinformatic testing of DNA sequence by exon splicing enhancer finder identified the sequence where the mutation lies as an exonic enhancer bound by SRSF1 (IgM-BRCA1) with a score of 2.03.
Conclusion: Our data suggest that this unique homozygous mutation might play a role in the pathogenesis of this patient. It is the first time to suggest the involvement of *PTBP1* gene in the etiology of a human genetic disease. Functional analysis should confirm the role of this mutation.

PS08.27

Expanding the phenotype associated with de novo mutations of the KDM5B gene

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Histone lysine methylation influences processes such as gene expression and DNA repair. Thirty Jumonji C (JmjC) domain-containing proteins have been identified and phylogenetically clustered into seven subfamilies. Most JmjC domain-containing proteins have been shown to possess histone demethylase activity toward specific histone methylation marks.One of these subfamilies, the KDM5 family, included four members. KDM5A has been pre-

viously demonstrated as involved in autosomal recessive intellectual deficiency (ID), while KDM5C has been reported mutated in nonsyndromic and syndromic form of X-linked ID with or without epilepsy, short stature, or behavioral problems. Interestingly, de novo loss-of-function and missense mutations in KDM5B were identified in patients with ID and autism spectrum disorder but also in unaffected individuals. Here, we report three novel de novo mutations in the KDM5B gene identified by whole-exome sequencing and confirmed by Sanger sequencing. A frameshift mutation c.2642_2643del CT was identified in a girl with severe epileptic encephalopathy, a splicing mutation c.808+1G>A was found in 17 year-old boy with delayed speech development, mild facial features including prominent nasal bridge, and highly arched palate, and moderate intellectual deficiency, and a missense mutation c.1642C>T (p.Leu748Phe) described as probably damaging by bioinformatics tools in a 5-year-old boy with developmental delay, autism features, and mild facial features. We will discuss the effect of mutation position (such as the deletion of the C-terminal part or mutation in the JmjC domain) and the influence of double/multiple events in other genes involved in neuronal function (such as PIGA, Shank3, and Shroom4 also found mutated in these patients) on the clinical phenotype.

PM08.28

GenIDA: a social network and database to inform on natural history of monogenic forms of intellectual disability and autism

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Intellectual disability (ID) has an incidence of 2% and overlaps with autism. It is characterized by a striking genetic heterogeneity that underlies a phenotypic heterogeneity in severity and in associated medical problems. Progress in genome analysis has allowed the identification of many recurrent CNVs and of more than 400 genes implicated in monogenic forms of ID/autism. An increasing number of genetic diagnoses are made in individuals with ID/autism but the genetic heterogeneity renders extremely difficult the determination of genotype-phenotype correlations and natural history. Symptomatic treatments for comorbidities are proposed with limited opportunities to assess their efficacies or potential adverse effects.
We have initiated the development of an alternative database model for specific genetic causes of ID/autism, organized in a social network format whereby most clinical information is entered by the family of the patient based on wide range questionnaires translated in different languages. Contacts between families affected by the same genetic cause is possible in an anonymous way, creating gene or CNV specific social-networks to which interested professionals could be associated, akin to disease specific patients associations. Anonymized summary data will be accessible to families and to professionals. We will present the structure and features of the GenIDA social network that is currently in its beta testing phase.
This innovative strategy to collect information on natural history and comorbidities of rare monogenic forms of ID/autism will promote families' empowerment and hopefully impact patients' care. The setting up of patient e-cohorts should favor international clinical studies.
Supported by USIAS and RaDiCo.

PS08.29

UNIGENE: Familial intellectual disability in Lithuanian patients

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Intellectual disability (ID) affects 1-3% of the population. Genetic mutations account for about half of the 60% of cases that are currently undiagnosed. The main aim of our Swiss and Lithuanian collaboration is to identify novel genome variants that cause congenital neurodevelopmental disorders. (UNIGENE project has received funding from Lithuanian-Swiss cooperation programme to reduce economic and social disparities within the enlarged European Union under project agreement No CH-3-ŠMM-01/04)
We enrolled 132 families with one or two affected members according to the following standardised selection criteria (IQ<70 or developmental delay (for children under 6 years old) in cases of congenital malformations of central nervous system, not previously described multiple congenital anomalies/ID syndromes, sporadic cases of unspecific ID, etc.) totaling 147 Lithuanian patients with ID. Patients were first screened by arrayCGH, and 17 patients/families (12%) were found to carry causative CNVs such as del10q22.1-q22.3, del6q16.1-q22.31, del17p13.2-p13.3 and mos dup8p11.22-q11.23. The remaining patients are currently being exome sequenced. The first results of these analyses include several potentially causative variants. In particular the genes OTOG, PKHD1L1, IFI44, GFI1 and SYTL3 were found to carry extremely rare variants in evolutionary conserved codons as compound heterozygote in families with two affected individuals. The validation of these findings through identification of more patients with mutations in these genes, in vivo complementation, as well as engineering of animal models is warranted and under way.

PM08.30

Further delineation of MEF2C-related autosomal dominant ID: 6 novel mutations and one intragenic deletion

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Haploinsufficiency of MEF2C, encoding a member of the myocyte enhancer factor 2 (MEF2) subfamily of transcription factors, was recently identified as the underlying cause of the 5q14.3q15 microdeletion syndrome, characterized by profound muscular hypotonia, severe intellectual disability, early-onset epilepsy and variable other anomalies. Including the first description of the phenocritical role of MEF2C in 2010, eleven patients with de novo point mutations or small indels were reported and only seven of them were described in detail.
We now report on six novel patients with de novo point mutations or small indels in MEF2C and one patient with an intragenic deletion identified by mutational screening of patients referred for Rett, Angelman or Pitt-Hopkins syndrome-like conditions. We provide functional characterization of the two detected missense mutations, both affecting the highly conserved MADS domain, by expression analysis of MEF2C and phenotypically related genes in vivo. In contrast to the generally observed absence of speech one of our patients harboring a novel missense mutation is the first reported patient being able to speak several single words at the age of 4 years functioning at the mild to moderate intellectual disability level (IQ55). In addition we observed absence of seizures in the second patient harboring a missense mutation indicating a milder phenotypic expression in some patients with missense mutations. In summary we expand the mutational spectrum of intragenic MEF2C defects, further delineate the associated phenotype resembling Rett and Angelman syndrome, and point to a milder clinical course in patients with missense mutations.

PS08.31

Clinical Exome Sequencing (CES) in Complex Syndromic Disorders

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Intellectual disability (ID) is defined by an IQ below 70 and observed in approximately 1.5 -2% of live births. Although ID can be caused by a variety of non-genetic factors, genetic traits contribute in 50% of the cases. 40% of these genetic cases can be elucidated by conventional diagnostic such as chromosomal analysis, aCGH, and fragile X testing, while 60% remain unsolved.In a pilot study, we used CES in 15 patients with ID in which conventional diagnostics yielded no causal aberration. Enrichment of target genes was performed using the Illumina TruSight One Sequencing Panel (4,813 genes associated with known clinical phenotypes). The analysis also included the unaffected parents (trio approach) and was performed on the HiSeq2500 Next-Generation Sequencing platform (Illumina, San Diego, CA, USA). Data analysis was performed using CLCbio Genomics Server (v5.5.2) (Qiagen, Hilden, Germany). 1,157 ID associated genes of the 4,813 enriched genes were chosen for further annotation and analysis. These evaluated genes were known from literature or databases to be associated with ID. In 5 of 15 patients, we were able to identify the likely cause of the ID. We detected mutations and possible pathogenic variants in different genes: ATRX, SPG20, UROC1, PMM2, EXOSC3. All detected mutations were confirmed by Sanger sequencing. Two were listed as pathogenic in the HGMD database and the patients' phenotypes were in accordance with the description in the literature. Therefore no further analysis was required. For the 3 other potentially pathogenic variants mRNA sequencing and metabolic testing were

performed.

PM08.32
NGS-based panel analysis of 20 children with intellectual disability and their healthy parents detects sporadic and compound heterozygous mutations

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Introduction: Intellectual disability (ID, IQ<70) affects up to 3 % of the general population. Until recently the underlying cause remained unknown in about half of the affected individuals. Next generation sequencing techniques help to elucidate the genetic background of ID. Apart from fragile X syndrome which is a common cause of ID (7%) and which has been part of the routine diagnostic work up for years, more than 1000 other genetic diseases for intellectual disability are known to date.
Material and Methods: Following in-solution enrichment of 526 ID-linked genes (exons and splice sites), we sequenced 20 female and male affected children and their parents (trio) in whom genomic imbalances and fragile X syndrome had been excluded.
Results: In three patients we detected de novo dominant disease-causing variants in the genes TUBA1A, MLL2 and GFAP. 6 patients were diagnosed with recessive childhood disorders inherited from their parents (ASPM, PCNT, PEX2, ATP2A2, CPS1 and ZFYVE26). Conclusion: In summary, the stepwise analysis of the index followed by carrier testing of the parents was replaced by immediate trio sequencing. This led to the direct identification of de novo variants and bi-allelic variants in 9 out of 20 cases. The data presented suggest that trio analysis may accelerate sequencing data interpretation and segregation analyses in families with cases of intellectual disability.

PS08.33
High throughput sequencing in molecular diagnosis of non syndromic intellectual disability. New mutations identified within 71 genes specifically selected for non syndromic ID.

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The technological improvements of the last years made possible considerable progresses in the knowledge of the etiology of Intellectual Disability (ID). However at present very little is known about the genetic heterogeneity underlying the Non-Syndromic form of ID (NS-ID). To investigate the genetic basis of NS-ID we analyzed 43 trios and 22 isolated NS-ID patients using a targeted sequencing (TS) approach. 71 NS-ID genes have been selected and sequenced in all subjects. We found probable pathogenic mutations in 7 out of 65 patients. The pathogenic role of mutations was evaluated through sequence comparisons and structural analysis was performed to predict the effect of alterations in a 3D computational model through molecular dynamics simulations. Additionally a genotype-phenotype correlation has been performed. This approach allowed us to find new pathogenic mutations with a detection rate close to 11% in our cohort. This result supports the hypothesis that many NS-ID related genes still remain to be discovered and that unlike the syndromic form, NS-ID is a more complex phenotype, likely caused by a deep and broad interaction between genes alterations and environment factors.

PM08.34
Searching for a novel gene responsible for mild to moderate intellectual disability syndrome

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Introduction: Intellectual disability is a neurodevelopmental disorder that is characterized by impaired intellectual functioning, and it affects 1-3% of the population. We investigated a consanguineous Pakistani family afflicted with mild to moderate mental retardation. The affected individuals were poor in reasoning, judgement and mathematical skills as compared to normal age mates. However, they could be trained for simple life tasks under supervision. Materials and Methods: Parents and one unaffected and four affected sibs were available for study. Disease locus was identified by linkage mapping using SNP genotype data. Whole exome sequencing was applied to find rare/novel variants at the locus. Results: The disease locus was an approximately 2-Mb region at 2p23.3 and yielded a multipoint LOD score of 3.7. Only homozygous missense *PTRHD1* p.Cys52Tyr mutation segregated with the disease in the family. All the affected siblings were homozygous for this mutation and the unaffected sister was not. None of the 98 control samples from the Pakistani population tested positive for the mutation. The mutation was predicted as damaging by bioinformatics tools. *PTRHD1* encodes a peptidyl tRNA hydrolase belonging to the PTH2 family. The protein has 140 amino acids whose sequence is totally conserved between human and chimpanzee, and 52Cys is conserved across all vertebrates. The gene is expressed in many organs, including the brain.
Conclusion: *PTRHD1* is a novel gene for mild to moderate intellectual disability. We are planning to continue the population screen and to perform gene expression assays in various parts of human brain.
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PS08.35
A patient with syndromic intellectual disability and features reminiscent of Cornelia de Lange syndrome due to a de novo mutation of EP300

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Rubinstein-Taybi Syndrome (RTS) is associated with growth retardation, intellectual disability, and distinctive facial features with broad thumbs and toes. Causative mutations in two genes, CREBP (RTS1) and EP300 (RTS2), have been described, accounting for approximately 75%, and 3% of cases respectively. The phenotype of the RTS2 patients is generally appreciated to be milder, though only 14 cases have been reported. Recently, Woods et al (AJMG 2013) reported a novel EP300 mutation in a patient with features that overlap Cornelia de Lange syndrome (CdLS).
We describe an 8 year-old girl referred for dyspmorphic features, short stature, microcephaly, and global developmental delay. Her mother had severe pre-eclampsia during her pregnancy. Trio-based whole exome sequencing identified a de novo mutation in EP300 (c.2713_2714delCC). Her physical features include full eye lashes, thin arched eyebrows, a low-hanging columella, mild micrognathia and hirsutism on her back and arms, but with normal thumbs and toes. Her features were reminiscent of mild CdLS. Psychometric testing revealed a full scale IQ in the borderline range; however subscale discrepancies were noted with relative strengths in verbal comprehension. Additional deficits were noted on assessment of her attention, executive function, visual perceptual processing, social perception and motor skills. Her outgoing personality and verbal strengths are reminiscent of William syndrome, and her educational and social struggles are significant. Our case further supports that there may be a broader phenotypic spectrum caused by mutations in this gene, and brings into question whether testing for EP300 mutations in patients with CdLS may be warranted.

PM08.36
Validation of *PTPN23* as an intellectual disability gene through identification of a second likely disease-causing variant

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Intellectual disability (ID) is an extremely heterogeneous disorder. In the last few years, whole exome sequencing (WES) has facilitated the identification of many new candidate genes in which mutations may lead to ID. However, often these results cannot be used in a clinical diagnostic setting, as no further mutations and/or patients have been described to provide a second independent validation of the initial results. Recently, Alazami *et al*. reported a missense variant in the *PTPN23* gene, c.3995G >T (p.Arg1332Leu), in one child with global developmental delay, epilepsy, brain atrophy, and some skeletal malformation.
Alazami concluded that this gene is a candidate gene for brain atrophy and global developmental delay. We performed WES in an 8-year-old boy presenting with global developmental delay and developmental regression, epilepsy, brain atrophy, microcephaly, ataxia and abnormal movements. In this analysis, we detected a homozygous variant, c.904A>G (p.Met302Val) in the *PTPN23* gene, which is highly conserved and predicted to be pathogenic by several but not all *in silico* programs. We observe a significant overlap of

symptoms between the two patients, and conclude that both mutations are probably causative.
Therefore, mutations in *PTPN23* are likely to be associated with an autosomal recessive form of ID with brain atrophy and epilepsy. Further symptoms such as skeletal malformation, regression and ataxia are reported in only one of the two patients and further studies are needed to precisely delineate the specific phenotype characteristics.

PS08.37

Title: Value of a diagnostic confirmation service by an accredited laboratory

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The explosion of Next Generation sequencing has produced an unprecedented amount of valuable data which has enabled potential characterisation and diagnoses in disease for a vast array of patients. Many of these findings are produced by research laboratories and confirmation in a diagnostic laboratory enables patients and their families access to publically funded healthcare resources through the NHS that would not otherwise be available to them including; disease support, family testing and prenatal diagnosis (PND).
We present an audit of our referrals from the areas of paediatric developmental delay and dysmorphology covering eighteen months of our diagnostic confirmation service. We review two of these cases in more depth and use them to highlight the benefits of the interpretation provided by using a diagnostic service; such as providing a classification for a phenotype or re-classification of a proposed diagnosis, both of which may have implications for patient management.

PM08.38

KBG Syndrome: A DDD front-runner?

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KBG syndrome (Omim #148050) is a rare genetic disorder caused by haploinsufficiency of the ANKRD11 gene. The phenotype has previously been described to include macrodontia of the mandibular central incisors, skeletal and craniofacial anomalies as well as intellectual disability. Many sources quote that the cognitive deficit and the clinical features in this condition can be mild, thus making it an underdiagnosed genetic condition.
The Deciphering Developmental Disorders (DDD) study aims to identify the underlying genetic cause in children with developmental delay and/or congenital abnormalities, where conventional genetic testing has failed. Exome sequencing of the first 1000 child and parent trios found several patients with likely pathogenic mutations in ANKRD11. This data suggests that KBG syndrome is not as rare or as benign as initially suspected which prompted a Complementary Analysis Proposal (CAP) to further explore the phenotype of this condition.
Questionnaires on detailed phenotypes were sent to the lead Genetic clinicians for all of the patients identified with ANKRD11 mutations through DDD so far. The data was amalgamated and photographs of the patients were obtained with parental consent.
Here we present a summary of the frequently reported clinical features, characteristic dysmorphology and genotype within this cohort of patients. Recommendations have been made about diagnostic clues and management strategies when this genetic diagnosis is suspected clinically.

PS08.39

Exome sequencing identifies three novel candidate genes implicated in intellectual disability

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Intellectual disability (ID) is a major health problem mostly with an unknown etiology. Recently exome sequencing of individuals with ID identified novel genes implicated in the disease. Therefore the purpose of the present study was to identify the genetic cause of ID in one syndromic and two non-syndromic Pakistani families. Whole exome of three ID probands was sequenced. Missense variations in two plausible novel genes implicated in autosomal recessive ID were identified: lysine (K)-specific methyltransferase 2B (KMT2B), zinc finger protein 589 (ZNF589), as well as hedgehog acyltransferase (HHAT) with a de novo mutation with autosomal dominant mode of inheritance. The KMT2B recessive variant is the first report of recessive Kleefstra syndrome-like phenotype. Identification of plausible causative mutations for two recessive and a dominant type of ID, in genes not previously implicated in disease underscores the large genetic heterogeneity of ID. These results also support the viewpoint that large number of ID genes converge on limited number of common networks i.e. ZNF589 belongs to KRAB-domain zinc-finger proteins previously implicated in ID, HHAT is predicted to affect sonic hedgehog, which is involved in several disorders with ID, KMT2B associated with syndromic ID fits the epigenetic module underlying the Kleefstra syndromic spectrum. The association of these novel genes in three different Pakistani ID families highlights the importance of screening these genes in more families with similar phenotypes from different populations to confirm the involvement of these genes in pathogenesis of ID.

PM08.40

Novel homozygous mutation in KPTN gene causing a familial intellectual disability-macrocephaly syndrome

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BACKGROUND: Recently, Baple et al. (Am J Hum Genet 2014, 94(1):87-94) described a large Amish pedigree where homozygous or compound heterozygous mutations in KPTN gene encoding kaptin protein resulted in a clinically distinctive syndrome consisting of macrocephaly, global developmental delay, behavioural abnormalities, and seizures (MIM 615637). Here we report the second case of KPTN-related syndrome in two Estonian siblings with novel homozygous KPTN mutation and similar phenotype.
CASE REPORT: The probands are 32-years-old brother and 24-years-old sister from Estonia. The parents are non-consanguineous, but were born in the same parish. The brother and sister have macrocephaly, with occipitofrontal circumference of 63 cm (+4.5 SD) and 60 cm (+4 SD) respectively. Their intellectual disability could be classified as moderate. The verbal abilities are more affected than the motor development in both siblings. Behavioural problems and a few episodes of seizures were present only in the brother.
METHODS AND RESULTS: Whole exome sequencing identified homozygous one-nucleotide frameshift duplication in KPTN gene (c.665dupA:p.Q222fs). Homozygosity of both affected siblings and heterozygosity of parents was confirmed by Sanger sequencing. SNP-array showed a 1.5 Mb homozygous stretch encompassing KPTN gene in both siblings.
CONCLUSIONS: With this report we confirm the pathogenicity of KPTN gene mutations and delineate the core phenotype of the novel autosomal recessive genetic syndrome. We also prove the hypothesis of the first describing authors that KPTN-related syndrome is not restricted to Amish population.
This work was supported by the Estonian Research Council grant PUT355.

PS08.41

Intragenic deletion of DOCK3 gene in a patient with dysphasia and intellectual delay

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DOCK3 (Dedicator of Cytokinesis 3) belongs to CDM family protein which regulates several biology process involved in the engulfment of apoptotic cells and in the cell migration. DOCK3 gene expression is restricted to the brain, notably in the occipital, frontal and temporal lobes where is the Wernicke's speech area implied in speech processing. To date, there is no well-defined clinical syndrome associated with DOCK3 gene mutation or deletion. The only clinical publication involving DOCK3 gene evoked a patient with attention deficit hyperactivity disorder-like phenotype and SLC9A9 and DOCK3 genes disruption.
Here, we reported a 11 year-old boy presenting with a phonological and synthaxic dysphasia, intellectual delay, without related dysmorphia, neither deafness nor visual acuity disorder. He was born at term, with these following parameters: weight 3730 g (75th percentile)- size 50.5 cm (50th percentile), HC 36 cm (75th percentile). His evolution was marked by mild cognitive delay, language impairment without motor delay. Cerebral MRI

was normal. His father and his three brothers have language impairment. We highlighted by array CGH (Agilent - 180K) an intragenic 156 kb deletion in DOCK3 gene located at 3p21.2. Familial exploration is in progress. This case is interesting because it could involve a new gene in language disorder associated with intellectual delay.

PM08.42

Identification of lncRNAs involved in neuronal development and intellectual disability

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Background
Recent studies have assigned important functions to lncRNAs in gene regulation and protein interactions. Since many of these lncRNAs emerged recently during vertebrate and primate evolution, a crucial role in the human brain is anticipated. Here, we aimed at identifying candidate lncRNAs associated with neuronal development and intellectual disability (ID).
Methods
A strategy was defined using publically available data regarding neuron specific histon modifications, REST binding & DNAse 1 hypersensitivity. This was complemented with extensive expression profiling of both coding and noncoding genes in seven nonneuronal tissues and eight brain samples. As a proof-of-principle, this approach was first applied to all RefSeq coding genes.
Results
Of the coding genes, 4040 met the criteria of having a neuron specific H3K4-me3 mark, REST binding and DNAse 1 hypersensitivity in the promoter region (filter 1) as well as a relatively high expression in at least one brain sample (filter 2). A significant enrichment of ID genes was noted in this list (178/4040) and in 65 genes, a GWAS hit for neuropsychiatric disorders was observed; indicating the power of this approach.
When applied to 32108 lncRNA transcripts (lncipedia 2.1), 2980 lncRNAs passed filter step 1. While the majority of lncRNAs have a relatively low expression, an enrichment of lncRNAs passing filter step 2 was seen (215/2980 versus 1360/32108). A GWAS hit was noted in one of these lncRNAs.
Conclusion
Using this integrated approach, we identified 215 interesting candidate lncRNAs for neuronal development. These lncRNAs will be functionally validated.

PS08.43

Partial rescue of RTT-like symptoms in Mecp2-deficient mice after administration of a self-complementary AAV9 (scAAV9) construct expressing a codon-optimized Mecp2 transgene.

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Rett syndrome (RTT) is an X-linked neurodevelopmental disorder primarily affecting CNS functions. Most RTT cases are due to mutations in the methyl CpG binding protein 2 (MECP2) gene, a global transcriptional modulator. There is currently no cure for the disease and drugs alleviating symptoms are the only available therapies.
Recently, two different research teams reported that gene therapy in the Mecp2-deficient RTT mouse model partially cured the disease (Gadalla et al 2013; Garg et al 2013). Although both studies showed a rescuing effect, the overall benefits of this therapy seemed to depend on the age at which the virus was administered, as well as the viral construct.
In order to try and improve vector delivery and expression, we designed a plasmid construct expressing a codon-optimized version of Mecp2 that was used to generate a scAAV9 virus. Thirty day-old Mecp2-deficient male mice were injected with the virus through the tail vein (2*1011vg/mouse). Post-mortem analysis was carried out in 60 day-old animals and the percentage of transduced brain cells was quantified by immunohistochemistry. Despite a low percentage of Mecp2-expressing cells (6-10% of WT levels), we did find an improvement in spontaneous activity (Open field) and sensorimotor coordination (Rotarod), as well as a decrease in apneas that are characteristic RTT symptoms (plethysmography).
These preliminary data indicate that even a low level Mecp2 expression can improve RTT symptoms in Mecp2-deficient mice. Further studies will aim at confirming these data and investigating the potential therapeutic effect of the same viral construct in female RTT mice.

PM08.44

Biallelic mutations in the Notch pathway regulator MIB1 in two brothers with syndromic intellectual disability and distinctive facial features

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Dominant mutations in the ankyrin domain and in the ring fingers domain of the E3 ubiquitin ligase MIB1, that promotes endocytosis of the NOTCH-ligand DELTA and JAGGED, have been linked to the etiology of familial non-compaction cardiomyopathy (NCCM) (Luxan et al 2013). In a large cohort of patients with unexplained intellectual disability (ID), de Ligt et al (2012) listed a de novo missense mutation in *MIB1* as candidate for ID. We observed a family consisting of three siblings born to healthy non-consanguineous Dutch parents. A girl died at the age of 9 months from complications of extra-hepatic bile duct atresia, abdominal situs inversus, polysplenism and intestinal malrotation. Two male siblings, at present 11 and 7.5 years old, presented with comparable problems consisting of failure to thrive, delayed motor developmental, microcephaly, intellectual disability, friendly behavior, delayed and nasal speech, large nose bridge and small nares, pointed chin, small mouth, high palate with teeth malocclusion, thin upper lip, featureless philtrum, large ears, short neck, fair complexion, kyphoscoliosis, barrel shaped chest. Cardiac screening of the eldest child at 2.5 years showed a slightly enlarged right ventricle, but was otherwise normal. Whole exome sequencing of DNA from both sibs showed two shared compound heterozygous changes in *MIB1*, one maternally inherited highly conserved missense variant,c.244G>A in the zinc finger domain and the other in the splice donor site for exon 3 (c.531+1G>T), adjacent to the Mib Herc2 domain. We hypothesize that biallelic recessive mutations in *MIB1* cause a severe and complex phenotype, including intellectual disability.

PS08.45

Detecting small Copy Number Variations, smaller than 400 kb, improves the diagnostic yield of CMA in intellectual disability

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Cytogenetic microarray (CMA) allows the elucidation of 15 to 20 percent of remaining unexplained cases of intellectual disability. A threshold of 400 kb is proposed to consider Copy Number Variants (CNV). Array use in diagnostic setting could detect smaller CNV mostly polymorphic generating numerous confirmations. Here, we propose to evaluate the diagnostic interest of small CNV (inferior to 400kb) in intellectual disability (ID). This collaborative study groups an Illumina SNP-array platform and an Agilent CGH-array platform for a total of 3330 patients explored for syndromic or isolated intellectual disability from 2011 to 2014.
Over 26,866 CNV detected, 22,683 (84.4%) were smaller than 400 kb. Among the 1440 CNVs considered as relevant, 850 (40.9 %) were smaller than 400 kb increasing by 70% the amount of necessary confirmation by another technique. We concluded that 44 of 605 (7.3%) of these CNVs (smaller than 400kb) were pathogenic. 36 were deletions which involved pathogenic gene in ID and 8 were duplications. It is noteworthy that, except a MECP2 duplication, all small duplications were either intragenic or probably truncating an ID gene possibly leading to haploinsufficiency.
To conclude, analyzing CNV smaller than 400 kb significantly increases the number of verification but enhances the diagnostic yield of 1.3 % in patients with ID, which is equivalent to 44 patients in our cohort. However, we propose not to consider small non-truncating duplications. While massively parallel sequencing techniques are becoming routine diagnosis, it seems preferable to detect as many pathogenic CNVs as possible using CMA.

PM08.46

Comparisons of Mcph1-deficient mouse embryonic fibroblasts

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Biallelic mutations in the MCPH1 gene are the cause of primary microcephaly (OMIM 251200) associated with premature chromosome condensation (PCC) in G2 phase and delayed decondensation post mitosis. MCPH1 encodes a multifunctional protein that was reported to be involved in brain development, DNA damage response and the regulation of chromosome

condensation.
In this study we investigated mouse embryonic fibroblasts (MEFs) from three different types of published Mcph1 knockout mice. We failed to detect a delay in G2 checkpoint release after irradiation, as shown in earlier experiments of our group using patient derived MCPH1-deficient cells. However, we did observe a flatter slope of increase of the mitotic rate of MEFs from the Mcph1 knockout mice compared to controls.
Furthermore, we compared the rates of different DNA repair focus formation after irradiation of cells with 1 Gy or exposure to hydroxyurea. Analysis of yH2AX and 53BP1 indicated up to three times higher rates of foci-positive cells in knockouts than in control cells. In contrast, RAD51 and RPA foci appeared at similar rates and with similar time courses in Mcph1-deficient as in control cells.
Finally, we prepared mitotic chromosomes to compare the levels of PCC. The rate of prophase-like cells (PLCs) of Mcph1-deficient MEFs was increased compared to those of normal control cells. The Mcph1 knockout MEFs showed PLC rates between 0.7-4.5%, whereas the controls showed rates only between 0.2-0.3%.
Our results suggest that the presence of Mcph1 is necessary for the early DNA damage response after irradiation and that the effect of Mcph1 deficiency is confined to mitosis.

PS08.47

Homozygous mutation in the eukaryotic translation initiation factor 2alpha phosphatase gene, *PPP1R15B*, is associated with extreme microcephaly, short stature, and developmental delay

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Protein translation is an essential cellular process initiated by the association of a methionyl-tRNA with the translation initiation factor eIF2. The met-tRNA/eIF2 complex then associates with ribosomal subunits and other translation factors, which together comprise the translational machinery. This process is regulated by the phosphorylation status of the α subunit of eIF2 (eIF2α); phosphorylated eIF2α attenuates protein translation. Here we report a consanguineous family with severe microcephaly, failure to thrive and intellectual disability in two siblings. Whole-exome sequencing identified a homozygous missense mutation, c.1972G>A; p.R658C, in PPP1R15B, a protein which functions with the PPP1C phosphatase to maintain dephosphorylated eIF2α in unstressed cells. The p.R658C PPP1R15B mutation is located within the PPP1C binding site. We show that patient cells have greatly diminished levels of PPP1R15B-PPP1C interaction, which results in increased eIF2α phosphorylation and resistance to cellular stress. Finally, we find that patient cells have elevated levels of PPP1R15B mRNA and protein, suggesting activation of a compensatory program aimed at restoring cellular homeostasis which is ineffective due to PPP1R15B deficiency. PPP1R15B now joins the expanding list of translation associated proteins which when mutated cause rare inherited diseases.

PM08.48

6q22.33 microdeletion in a family with intellectual disability, variable major anomalies and behavioral abnormalities

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Interstitial deletions on the long arm of chromosome 6 have been described for several regions, such as 6q16, 6q22.1, and 6q21q22.1, and with variable phenotype such as intellectual disability/developmental delay, growth retardation, major and minor facial anomalies. An overview is given by Rosenfeld et al., reviewing twelve reported cases of the literature and proposing phenotype-genotype correlation [Rosenfeld et al. 2012]. However, an isolated microdeletion of the sub-band 6q22.33 has not been described so far and thus, no information is available concerning the specific phenotype associated with such a copy number variation.
Here we define the clinical picture of an isolated 6q22.33 microdeletion based on the phenotype of six members of one family with loss of app. 1 Mb in this region. Main clinical features included mild intellectual disability and behavioral abnormalities as well as microcephaly, heart defect, and cleft lip and palate. The mother attended supportive school; however she did not require a legal guardian and lived independently with regular social support regarding the childcare.References:Rosenfeld JA, et al. 2012. Genotype-phenotype correlation in interstitial 6q deletions: a report of 12 new cases. Neurogenetics 13(1):31-47

PS08.49

The costs and benefits of clinical genomic sequencing for monogenic disorders

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Genetic disorders affect a large proportion of the population and result in substantial economic and social burden. The rapid advances in genome sequencing have created an opportunity to rapidly and more universally identify disease-causing gene mutations, facilitating more efficient diagnosis and improved disease management and prevention. Here we have compared the costs of disability resulting from gene mutations with the potential health and social benefits of implementing whole genome testing for the detection of significant monogenic diseases. We found that the annual costs of intellectual and physical disability are substantial with average costs ranging from about $40,000 per annum for children with an intellectual disability to over $400,000 for young adults with severe intellectual disability. The aetiology of many conditions associated with intellectual and/or physical disability can be identified through clinical genomic sequencing at a current cost (including analysis) of ~US$10,000, although that cost should decline rapidly with continued technological advances and especially the development of well-curated genotype-phenotype correlation databases. Different applications of clinical genomic sequencing offer varying abilities to avoid cases of disability with greater benefits likely to be realized the earlier in the reproductive cycle testing is offered. The potential to mitigate even a fraction of the costs and social impacts associated with disability through clinical genomic sequencing makes a compelling socioeconomic case for consideration of implementation of such testing as well as the construction of supporting national and international databases.

PM08.50

Whole gene duplication of SCN2A and SCN3A is compatible with normal intellectual development

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Duplications at 2q24.3 encompassing the voltage-gated sodium channel gene cluster are associated with early on-set epilepsy. All cases described in the literature also presented with different degrees of intellectual disability, and has apart from the sodium channel gene cluster also involved neighboring genes.
Here we describe a family including a mother and two daughters, all presenting with a duplication involving only SCN2A, SCN3A and the 5'-part of SLC38A11. All three had a normal cognitive development and infantile epilepsy with a spontaneous remission after one year. This suggests that an extra copy of SCN2A and SCN3A have an effect on epilepsy pathogenesis, which might be similar to what is seen in patients with SCN2A mutations. However, the number of copies of SCN2A and SCN3A does not seem to have an effect on cognitive outcome.

PS08.51

The power of next generation sequencing in identifying atypical presentations of known OMIM genes: the exemple of CUL4B

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Intellectual disability (ID) has an estimated prevalence of 2% in the general population and is more common in males. Mutation in CUL4B, a gene encoding for a member of cullin-RING complex, is known to cause Cabezas syndrome corresponding to a X-linked ID associated with aggressive outburst, seizures, hypogonadism and dysmorphism. To date, 35 males patients are reported with mutation in CUL4B, all of the propositus were diagnosed after the age of normal puberty. We report here 3 new patients of 2 families with CUL4B mutations, diagnosed with Next Generation Sequencing (NGS) at a younger age (4 to 19 years) and presenting with atypical features such as sensory neural deafness, iris heterochromia and patchy depigmentation of the skin (suggesting a Waardenburg syndrome), hypertrophic cardiomyopathy, or medullar ischemia in early infancy. All of them presented with mild to severe psychomotor delay, macrocephaly and facial dysmorphism. Short

stature, obesity, kyphosis, seizures and hypogenitalism were found in 2 out of 3 patients after reverse phenotyping. Due to these confounding factors, the diagnosis of Cabezas syndrome has not been clinically raised and needed NGS. Two novel truncating CUL4B mutations, were found, , inherited from the healthy mother of the 2 brothers in family 1, and de novo in the 3rd patient. In conclusion, the use of NGS and reverse phenotyping allowed to establish the diagnosis in these atypical presentation of the Cabezas syndrome.

PM08.52

Unexpected molecular diagnoses of intellectual disabilities: making headway with the Trusight One sequencing panel

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Recent advances in genetic technologies provide new opportunities to elucidate genetic defects. The introduction of NGS methods in the diagnostic of intellectual disabilities (ID) led to the identification of 20 to 50 % supplementary disease causing variants.
We applied the Trusight One technology (Illumina) which contains the 4813 genes already involved in human pathology.
We studied a series of 36 patients with ID who have been extensively investigated without diagnosis.
From our first results, this method allowed us to disclose diagnoses that were unexpected owing to:
- the mild phenotype of a known syndrome, well exemplified by a sibpair with apparently non syndromic ID carrying an ATRX mutation
- the limited knowledge about very rare and/or poorly described phenotypes or the lack of specificity of a given disorder, as illustrated by a patient with a GRIA3 mutation
- the limited knowledge about the early phenotype of some progressive diseases with developmental delay, as exemplified by a young patient with ID but without typical brain MR images carrying PANK2 mutations.
Among NGS approaches in ID, Trusight One technology is an interesting tool for the diagnosis of known but rare diseases.

PS08.53

Mutation in NRAS in familial Noonan syndrome - Case report and review of the literature

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Noonan syndrome (NS), a heterogeneous developmental disorder associated with variable clinical expression including short stature, congenital heart defect, unusual pectus deformity and typical facial features, is caused by activating mutations in genes involved in the RAS-MAPK signaling pathway.
We present a clinical and molecular characterization of a family with Noonan syndrome. Comprehensive mutation analysis of *NF1, PTPN11, SOS1, CBL, BRAF, RAF1, SHOC2, MAP2K2, MAP2K1, SPRED1, NRAS, HRAS* and *KRAS* was performed using targeted next-generation sequencing. Result revealed a recurrent mutation in *NRAS*, c.179G>A (p.G60E), in the index patient. This mutation was inherited from patient's father, which also showed signs of NS.
Neither of the affected individuals in this family presented with juvenile myelomonocytic leukemia (JMML), which together with previously results suggest that the risk for NS individuals with a germline *NRAS* developing JMML is not different from the proportion seen in other NS cases. 50% of NS individuals with an *NRAS* mutation present with lentigines and/or Café-au-lait spots, demonstrating predisposition to hyperpigmented lesions in *NRAS*-positive NS individuals. Moreover, the affected father in our family presented with a hearing deficit since birth, which together with lentigines are two characteristics of NS with multiple lentigines (previously LEOPARD syndrome), supporting the difficulties in diagnosing individuals with RASopathies correctly. The clinical and genetic heterogeneity observed in RASopathies is a challenge for genetic testing. Advantages of next-generation sequencing allows screening of large number of genes simultaneously, which will have an important impact on the correct diagnosis, prognosis and treatment of these patients in the future.

PS08.55

Similar orofacial phenotype in a family with Xq24-Xq25 duplication

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Mandibular prognathism is a common dentofacial phenotype with a substantial genetic component, however, few susceptibility loci have been mapped.
Mandibular prognathism is a feature of the XXY, XXXY, and XXXXY syndromes and of interest is the progressive increase of this feature as the number of X chromosomes increases.
We refer a family, mother and two adult boys with the same orofacial morphological pattern, for clinical and craniometric assessment and for cephalometric analysis.
The index case - male of 30 years old with intellectual disability and obesity, dysmorphic features with triangular shaped face, short palpebral fissures, prognatism, macrostomia, and low set ears. Surprisingly, the brother of 32 years old and the mother of 60 years old presented the same orofacial features, associated with milder intellectual disability.
Given the intellectual disability, genetic testing was carried out. The karyotype was normal. As regards the array-CGH analysis, a Xq24-Xq25 ChrX:115.568.872-126.991.548 bp, microduplication was found, confirmed through quantitative Real Time PCR. BAC-FISH was done for all 3 family members and it revealed that the duplicated segment was located on chromosome X and it was likely to have a direct orientation. On extraoral examination, they presented oval face with appearance of long face due to the lower facial level predominance, through a short and flat philtrum, sharp chin, retrusive concave facial profile.
At craniometry they presented hyperbrachicephalic type according to the cranial index, hyperleptoprosop facial type according to the facial morphology index, and leptorrhine nasal type. Lateral cephalometric analysis showed skeletal class III malocclusion with prognathism.

PM08.56

Exploring possible genetic heterogeneity in Pitt-Hopkins syndrome combining whole-exome and targeted next-generation sequencing.

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Pitt-Hopkins syndrome (PTHS) is characterized by ID, typical facial gestalt, and additional features, including breathing abnormalities. It is generally caused by the haploinsufficiency of TCF4 gene. However, no mutation can be identified in a relevant number of patients with an apparently typical phenotype.
We developed a clinical checklist based scoring system to select patients presenting with a phenotype that is highly suggestive of PTHS. We tested for pathogenic variants in TCF4 a total of 200 patients referred with a clinical suspect of PTHS, of whom 25 had a proven mutation in TCF4. Based on our score system we selected 75 subjects with a score >10 and normal TCF4 to be studied in search of the disease causative variant. Patients were further grouped into categories with homogenous phenotypes according to their score and the presence of additional clinical features.
Aiming to explore the possible genetic heterogenity underlying cases with PTHS-like phenotype but with negative molecular tests, a small group of these subject (n=4) already underwent whole exome sequencing (WES) analysis. It allowed us to identify a set of variants in shared genes to be considered for further validation.
Additionally, we have been performing targeted next generation sequencing on the remaining patients, by using a custom panel including both genes responsible for diseases that are to be considered in differential diagnosis of PTHS, and genes resulting from exome sequencing data filtering and prioritization.
Preliminary results are presented and discussed.
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PS08.57

Heterozygous deletion of 4 exons of the PTPRT gene confirms its contribution to intellectual disability.

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Understanding of the genetic basis of intellectual disability (ID) has been increased in recent years. The use of sensitive pangenomic techniques as aCGH and SNParray contributes to discover new genes associated with syn-

dromic and non-syndromic ID.
We reportan 18-month-old Turkish child with a psychomotoric developmental disorder who presents with unstable sitting, severe hypotonia and speech delay but without dysmorphisms. SNP array analysis identified a „de novo" 185 kbp deletion on chromosome 20q12 encompassing a deletion of 4 exons of a single coding gene, named PTPRT (Protein-Tyrosine Phosphase,Receptor Type T). The PTPRT gene entry in OMIM database (608712) includes a single variant of unknown significanceTHR1365MET [dbSNP:rs199947379].
This variant was revealed due exome sequencing in 3 Dutch sibs with severe intellectual disability, published by Schuurs-Hoeijmakerset al. (2013). Authors noted that the PTPRT gene is expressed in the brain or in neuronal tissue and encodes a transmembrane receptor of the protein tyrosine phosphatase family, which are important proteins in signal transduction. They described a complex phenotypein 5 affected sisters including behavioral problems, microcephaly, congenital heart defects, short stature, and diaphragmatic herniation. The combination of a heterozygous missense variant and a heterozygous intronic deletion of 150 kb suggests an autosomal recessive pattern of inheritance.
In our patient we considered no heart defects and diaphragmatic herniation. Due to age behavioral problems have not been evaluated until today.
In summary, we report a case of ID associated with a heterozygous deletion of the PTPRT gene, confirming a PTPRT haploinsufficiency, which contributes to intellectual disability.

PM08.58
Mutations in QARS, encoding glutaminyl-tRNA synthetase, cause normocephalic intellectual disability and seizures - expansion of the phenotype

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Intellectual disability (ID) and seizures are common characteristics of numerous neuro-genetic syndromes. Recessive loss of function mutations in QARS, encoding Glutaminyl-tRNA Synthetase, were recently described in 2 families as a cause of ID with intractable seizures in infancy, progressive microcephaly , and atrophy of the cerebral cortex, cerebellar vermis and cerebellar hemispheres (Zhang et al 2014).
We report two children with ID and seizures, born to first-cousin parents. Both were born at term, with normal birth weights. The affected boy had multiple-type seizures (atypical absence and drop attacks) from age 3 months, while his sister had mild atypical absences from age 6 years. Both siblings (currently 11.5 years and 10 years old respectively) have severe ID with expressive language impairment, but are both normocephalic with normal brain imaging and mild dysmorphic features.
Whole Exome sequencing revealed 5 possibly damaging coding variants homozygous in both affected children and heterozygous in the parents: 4 missense variants and one stop loss mutation. Only one was in a gene associated with a neurological phenotype: QARS c.858C>A; p.Phe286Leu, not reported in 1000 genome, EVS and ExAC. This missense mutation is predicted to be damaging by Polyphen (0.999). Two unaffected sibs were heterozygotes for the QARS mutation. Functional studies of this mutation will be presented.
Glutaminyl-tRNA Synthetase is a ubiquitously expressed class-I aminoacyl-tRNA synthetase. Our results suggest QARS mutations cause a wider spectrum of functional brain abnormalities, and should be considered as a candidate gene for non-syndromic ID with seizures.

PS08.59
RBFOX1 intragenic deletions detected by array-CGH - 6 new cases with neurological phenotype

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Introduction: In the last couple of years, it has become evident the importance of whole genome array-CGH in the screening of de novo microdeletions or duplications in patients with phenotypic features of mental retardation (MR) and autistic spectral disorders (ASD). Copy number variations (CNV) in RBFOX1, also known as A2BP1, have been associated with human neurodevelopmental disease, including autism spectrum disease.
This gene, spanning 1.7 MB on chromosome 16p13.3, is one of the largest genes in the human genome, responsible for encoding splicing regulatory factors, specifically expressed in neurons and muscles, and having a regulatory action in alternative splicing of a large tissue-specific gene networks.
Materials and methods: Oligonucleotide array-CGH analysis, using an Agilent 4x180K platform, was performed in 1200 patients with mental retardation, autism spectrum disorders and congenital anomalies.
Results: In our cohort, we identified 6 cases with intragenic deletions in RBFOX1, 4 of them with additional CNV in other chromosomes. These CNVs are located at 16p13.3, extended in a 1,2Mb region between 6,087,983-7-,207,012 (GRCh37/hg19), which corresponds to intronic regions in 5 out of 6 patients. Five of these patients have an inherited deletion, from an unaffected progenitor, and one has an unknown inheritance pattern.
Conclusions: The phenotypes of all these children have in common, as widely described, neurological alterations. The rearrangements identified lead to a reduction in RBFOX1 expression, corroborating the importance of RBFOX1 haploinsufficiency in this phenotypes. The continuing follow-up of these patients and similar cases can help our understanding on the phenotypic diversity of these patients.

PM08.60
Mosaicism for a start codon mutation in MECP2 in a male with clinical features of Rett Syndrome: phenotypic description and review of the literature.

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Rett Syndrome (RTT) is an X-linked neurodevelopmental disorder caused by mutations in the methyl CpG-binding protein 2 (MECP2) gene at locus Xq28, which presents with both classic and variant phenotypes in females. In males, mutations similar to those that cause RTT in females are usually lethal; however, somatic mosaicism for classic RTT mutations or less deleterious, nonsynonymous mutations result a spectrum of neurological features reminiscent of classic RTT in females. In this case, an 8-year-old male presented with clinical features of RTT including: microcephaly, Chiari I malformation, neurodevelopmental regression, seizures, bradykinesia, apnea and stereotyped midline hand movements. MECP2 analysis identified both a normal sequence and a c.1A>T (p.Met1?) mutation in peripheral blood and fibroblasts with normal dosage by multiplex ligation-dependent probe amplification (MLPA). This has only been reported in females with classic RTT. Somatic mosaicism for classic RTT mutations has rarely been reported in males, however, this is the first known case involving the start codon of MECP2, p.M1. Subsequent analysis by array comparative genomic hybridization (aCGH) did not identify copy number changes in MECP2 but did pick up a 172 kb deletion of Xq21.31, which is considered unlikely to contribute to the patient's phenotype. The unbalanced heterozygous mutation suggests somatic mosaicism. Although a small duplication cannot be ruled out given the limitations of our array and MLPA probes, this is felt to be less likely to be the mechanism. The phenotypic presentation of this patient is discussed along with a review of the current literature on males with RTT.

PS08.61
Syndromic X-linked intellectual disability segregating with a missense variant in RLIM

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We describe a three generation Norwegian family with a novel X-linked intellectual disability (XLID) syndrome characterised by subtle facial dysmorphism, autism and severe feeding problems. By exome sequencing we detected a rare missense variant (c.1067A>G p.(Tyr356Cys) NM_183353.2) in the RLIM gene, in two affected male second cousins. Sanger sequencing confirmed the presence of the variant in the four affected males and in the three carrier mothers available for testing. The variant was not present in 100 normal Norwegian controls, has not been reported in variant databases and is deleterious according to in silico prediction tools (SIFT, Polyphen and MutationTaster). The clinical phenotype and the variant co-segregate, with a LOD score of 3.0 for linkage to the shared region (36.09 Mb). No other shared rare variants on the X-chromosome were detected, and all female carriers had an extremely skewed X-inactivation pattern.
RLIM encodes ring zinc finger protein 12 (RNF12), an ubiquitin ligase which is essential for X-inactivation in mice and which acts as a co-regulator of a range of transcription factors, particularly those containing a LIM homeo-

domain. Tyrosine in position 356 in RNF12 is located within a highly conserved domain essential for binding such transcription factors. Expression of RNF12 is widespread during embryogenesis, and particularly high in the outer layers of the cerebral cortex.
The detected variant likely disrupts an essential function of RLIM in neurodevelopment, and raises the possibility of important, previously unknown roles for this ubiquitin ligase.

PM08.62
Identification of an inherited splicing SCN8A mutation in a family with intellectual disability, autism, ataxia and seizures by whole exome sequencing

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Whole exome sequencing (WES) can identify causes of abnormal clinical phenotypes. We report a 15-year-old boy and his 9-year-old brother with normal karyotypes and SNP array findings who showed moderate intellectual disability (ID), autism and speech delay. The older boy had abnormal EEG and showed absence seizures since the age of 9 years. The younger boy presented ataxia at the age of 5 years. Their mother showed mild ID, as did the maternal grandmother and a maternal uncle.
WES of the brothers and their mother identified a heterozygous splicing *SCN8A* mutation (NM_014191, c.1134+1G>A) which was absent in all exome databases. It was confirmed using Sanger sequencing in both boys, their mother, grandmother and uncle, and predicted to disrupt the splice donor of intron 9, with exon 9 skipping being one of the likely outcomes. Sequencing of lymphocyte cDNA of the carriers confirmed this aberrant transcript leading to frameshift and premature termination (p.G331GfsX11). The interpretation of this variant transcript is however complicated by its presence also in normal controls. Other variant transcripts and differences in transcript ratios are under study.
Multiple *SCN8A* mutations have been reported, mostly *de novo* missense variants in patients showing predominantly seizures, while one truncating variant segregated in a family with a broader spectrum of cognitive and behavioural deficits. Our findings may support the notion that while missense *SCN8A* mutations causing increased channel activity can be associated mainly with seizures, heterozygous loss-of-function mutations may predispose to a slightly different phenotype.
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PS08.63
Single genes deletions/duplications detected by array-CGH: diagnostic relevance and suggestions for new disease genes.

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During the application of array-CGH to the diagnosis of syndromic forms of intellectual disability (ID), a total of 39 Copy Number Variants (CNVs) limited to single genes were observed, with size ranging from 85 Kb to 1.3 Mb. They accounted for 1.5 % of cases (38/2600). Results were confirmed by a second independent experiment. Sequencing of the candidate gene on the normal homologous was performed in some cases, with normal results.
Based on gene content analysis and on biological properties of the genes, a total of 5/38 CNVs, encompassing FMR1, VPS13B, SCN1A, MEF2C and APC, respectively, were considered pathogenic, with clinical presentation in carriers recapitulating the expected phenotype. A total of 10/38 CNVs (nine recurrent deletions and one duplication, encompassing GPHN, AUTS2, CTNNA3, SHANK2, and CNTNAP2) were likely pathogenic variants, previously described as characterized by incomplete penetrance and variable expressivity, ranging from ID to ADS.
A total of additional three CNVs define new genetic conditions, most likely, never reported before. They include 1) duplication of ZIC3 in a male subject with complex malformations resembling the oculo-auriculo-vertebral spectrum, 2) partial NFIB deletion associated with trigonocephaly and ID, 3) partial CDC42SE2 deletion associated with periventricular heterotopia. The remaining 20 CNVs
were considered of uncertain significance.
These observations focus on rare small CNVs limited to single genes that should be analysed by direct sequencing in additional subjects with similar clinical presentations.

PM08.64
Homozygous missense mutation in SNIP1, encoding Smad Nuclear Interacting Protein 1, associated with severe autosomal recessive neurodevelopmental disorder

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Introduction: Neurodevelopmental disorders are a highly heterogeneous and diverse group of genetic or acquired conditions that are responsible for brain or central nervous system dysfunction. Although many genes responsible for these conditions have been discovered, the aetiology and disease pathogenesis of most forms remains poorly understood.
Materials and Methods: Using a combination of autozygosity mapping and whole exome sequencing (WES), we studied an autosomal-recessive neurodevelopmental disorder with seizures, craniofacial dysmorphism and other features found among the Amish community.
Results: Whole-genome mapping of family members from two sibships using HumanSNPCyto-12 DNA Analysis BeadChip revealed a homozygous region on chromosome 1p34 common to the three affected children. Exome sequencing of one affected child identified a novel homozygous mutation in SNIP1 c.1097A>G (p.Glu366Gly) as the likely causative variant. The variant cosegregated with the disease phenotype in the families investigated as well as a further seven affected individuals from four other nuclear Amish families. Five heterozygous carriers of the mutation were identified among 350 Amish controls, which is not unexpected in this genetic isolate. Mutation pathogenicity was predicted through five in-silico programs.
Conclusions: Our findings define the clinical spectrum associated with biallelic mutation in SNIP1, which we show to be the likely cause of the severe neurodevelopmental disorder in these families. Assessing the functional outcomes of the missense mutation in the encoded protein and its regulatory function will expand our knowledge about the essential role of SNIP1 in growth and development of the brain or central nervous system.
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PS08.65
Deciphering the biochemistry of Snyder-Robinson Syndrome: Metabolomics identifies a new biochemical marker in patients affected by Snyder-Robinson syndrome (SRS)

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Snyder-Robinson syndrome (SRS) is a probably underdiagnosed X-linked disorder characterized by intellectual disability, dysmorphic face, muscle hypotonia, kyphoscoliosis, osteoporosis, seizures, and speech and gait abnormalities. SRS is caused
by mutations in spermine Synthase (SMS), but diagnosis of SRS has been hampered by lack of a specific biomarker that can be measured in body fluids or native cells. The balance of spermine and spermidine is maintained by SMS and is crucial for proper chromatin structure, ion channel regulation, transcription and translation.
We established the diagnosis of SRS by clinical exome sequencing in two novel monozygotic twin patients and subsequently demonstrated an abnormal spermine/spermidine ratio in cultured lymphoblasts. The novel Arg130Cys mutation is predicted to diminish enzymatic activity by decreasing dimer stability and affecting the structure of the adjacent spermine binding site. The third SRS patient investigated was published previously (Mastrangelo et al. 2013). Differential expression profiling of metabolites in the SRS patients revealed a high peak that was absent in controls. After identification of the SMS mutation, the abnormal peak could be readily identified as N-acetylspermidine, a polyamine intermediate product involved in spermine and spermidine metabolism. Thus we conclude that spermine synthase deficiency leads to intracellular accumulation of spermidine, which is converted to N-acetylspermidine and exported from the cell. Therefore targeted or untargeted plasma analysis of N-acetylspermidine represents a powerful screening marker for SRS relying on a single blood plasma sample.
Our study demonstrates the great potential of a combined genetic and me-

tabolomic approach and provides further insight into the metabolic dysregulation of SRS.

PS08.67

Compound heterozygous mutation in SZT2 gene in a patient with macrocephaly, epilepsy and severe intellectual disability

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We report on a family included in a trio exome sequencing study performed on 250 patients with intellectual disability.
The 15 year old boy is the first and only child of healthy, non-consanguineous German parents, born at term after a pregnancy complicated by uterus myomatosus and gestosis. Birth measurements were normal but green amniotic fluid was noted. The boy walked independently with 2 ½ years and never learned to speak and he is not toilet trained. Seizures started with 4 years of age and could be treated effectively. Agitated behavior and autistic features needed medication. Brain MRI revealed reduced temporal gyri on the left. The patient attends a special school and behaves like a toddler. Body measurements were normal throughout life, but head circumference was above the 97th centile. The patient has a high and broad forehead, prominent lips and small hands and feet.
Trio exome sequencing revealed a maternally inherited frame-shift mutation (c.841delC, p.(Gly281Ser*fs**33)) and a paternally inherited missense mutation in *SZT2* gene (c.9787G>A, p.(Asp3263Asn)). The possibly damaging missense mutation may lead to a residual protein function and cause a milder phenotype resembling the three brothers described by Falcone et al (2013) who are homozygous for a 3-bp in-frame deletion. Patients with homozygous or compound heterozygous truncating mutations as described by Basel-Vanagaite et al (2013) show severe infantile encephalopathy with epilepsy. This sixth patient broadens the phenotypic spectrum caused by mutations in *SZT2* gene showing an intermediate clinical course compared to the patients published to date.

PM08.68

Targeted molecular diagnosis of intellectual disability with or without autism: update from 217 to 275 genes and 100 to 300 patients confirms a 20-25% diagnostic efficiency and highlights genes recently identified or recurrently mutated

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We have recently reported the results of targeted sequencing (TS) of 217 genes implicated in intellectual disability in 106 patients (mostly males) with ID and no diagnosis after CGH array and phenotype-driven testing of candidate genes (Redin et al. J Med Genet 2014). The diagnostic yield after stringent evaluation was 26% (23% for sporadic ID cases). We have now extended this using an improved panel of 275 genes, and have tested another 200 patients. Our current results confirm a diagnostic yield of 20 to 25%. Our results indicate that de novo mutations in few genes, such as DYRK1A or TCF4, are found recurrently in ID patients and may account each for 1-2% of cases (ie not far from the incidence of fragile X). The update of our gene list allowed us to identify mutations in very recently identified ID genes, such as NAA10, TBR1, POGZ, TRIO, ZBTB20 (Primrose syndrome). We also report the first non-consanguineous and compound heterozygous case of AP4S1 ID with spastic paraplegia. Whole exome sequencing (WES) performed for fifteen individuals with no mutation identified after targeted sequencing (TS) did not reveal any obvious mutation in other genes not included in the panel. The large decrease in the depth of coverage obtained for WES compared to TS (60X vs 300X) made CNV analysis (with depth-of-coverage-based methods) and variant detection less efficient for our genes of interest. Therefore, until prices fall and coverage increase, targeted sequencing of candidate genes remains a useful method for routine diagnosis of intellectual disability.

PS08.69

A second family with THOC6 mutations and syndromic intellectual disability

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Exome sequencing is a new technology allowing the identification of the molecular bases of genetic conditions in patients without clinical diagnosis. We report the case of a 6-year-old boy with intellectual disability (ID) and severe language delay, hyperactivity, microcephaly, genital malformation with undescended testes, and peculiar dysmorphic features. MRI showed a dysplastic corpus callosum. Classical investigations, including array-CGH were negative. Exome sequencing found compound heterozygous mutations in THOC6 (p.Tyr45* and p.Gly190Glu). THOC6 is a member of the THO/TREX complex which is involved in coordinating mRNA processing with mRNA export from the nucleus, highly expressed in the midbrain and eyes. To date, a unique consanguineous Hutterite families comprising 4 affected children with common ancestors have been described with a THOC6 homozygous mutation. The patients have distinctive facial features, microcephaly, developmental delay, and possible heart and genitourinary malformations. Reverse phenotyping confirmed the presence of similar facial features, including tall forehead with high anterior hairline, deep-set eyes with short, upslanted palpebral fissures, long nose with low-hanging columella, and thick vermilion of the upper and lower lip. Other common features included the severe speech difficulties and the presence of genital malformations. Additional cases are requested to consider this gene as an undoubtful disease-causing gene.

PM08.70

Fast and effective genome editing to study dominant de novo mutations: the WDR45 example

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Increasing Trio sequencing for intellectual disability delivers more and more potentially disease causing de novo DNA variants. This situation necessitates an effective validation tool. We present an effective genome editing approach based on embryo microinjection of TALENs. We chose to generate a mouse model for the X-linked dominant form of NBIA (neurodegeneration with brain iron acculumation), caused by de novo loss-of-function mutations in WDR45. In humans, the course of disease is two-staged with developmental delay and intellectual disability in childhood and a second phase of rapid neurological deterioration characterized by parkinsonism and dementia occurring in early adulthood with characteristic neuroimaging findings such as hypointense signals in substantia nigra and globus pallidus. Microinjection of TALEN mRNA into oocytes resulted in 19% mutated founders (5/26). Mutations were deletions between 10 and 57bp with a frameshift in 4/5 mutations. From the in situ design and delivery of TALENs (~1month), over in vitro testing in cell culture to in vivo injection into embryo's (~2months) until the genotyping of founder mutants it took 4 months. The murine Knock-Out proved to not necessarily be de novo; therefore we chose a mouse harboring a 20bp deletion in Exon 2 for further breeding. Investigation of nine month old male brains (n=8) revealed numerous degenerated neurons and large axonal spheroids, clear signs of neurodegeneration in medulla oblongata, cerebral cortex and thalamus of mutant animals only. Furthermore, a neurobehavioral screen of 21 animals at the age of one year showed motor impairment (Balance Beam, p=0.004; Inverted Grid, p=0.018; Open-field, p=0.014).

PS08.71

A new ILRAPL1 point mutation: clinical report of a X-linked mental retardation family.

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IL1RAPL1 (interleukin-1 receptor accessory protein-like 1) located at Xp21.3-21.2 has been shown to be deleted in X-linked mental retardation patients with contiguous gene syndrome. Rarely, intragenic deletions or mutations have been identified. Non-specific intellectual disability, behaviour impairment with autistic spectrum disorder (ASD) and mild dysmorphism are actually recognized to be associated with IL1RAPL1 mutations.
IL1RAPL1gene comprises 11 exons and harbors 3 extra-cellular Ig-like domains (NM_014271.3). IL1RAPL1 protein does not bind interleukin-1 but is regulating the formation of synapses of the cortical neurons. IL1RAPL1 forms with the protein tyrosine phosphatase rho (PTPrho) a complex inducing pre and postsynaptic differentiation (Hayashi et al., 2013).
So far, very few papers reported family with XLMR caused by point mutations in IL1RAPL1 gene (Ramos-Brossier et al., 2014). We report here a family

with XLMR with two brothers as probands, their mother and sister carrying a new point IL1RAPL1 mutation c.1054C>T (p.Arg352*). The mutation was identified after X-chromosome analysis by next generation sequencing technique. This new mutation lying in exon 8 induces a premature stop codon. The resulting truncated protein is predicted to lack part of extracellular domain, transmembrane domain and the entire cytoplasmic domain. To our knowledge, this substitution is not yet described in the literature but its pathogenic character is highly likely.
Interestingly, in contradiction with the so far published data, all the female carriers were found with mild learning disabilities, and some behaviour impairments without any X-skewed inactivation detected at the level of lymphocytes DNA.

PM08.72
Recurrent microduplications at Xp22.31 are not sufficient to convey a disease phenotype

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The pathogenicity of Xp22.31 duplications is still controversial. Recent studies have suggested a possible causal relationship of this genomic rearrangement with an abnormal phenotype, indicating that it may be a risk factor for intellectual disability.
In an effort to clarify the significance of these duplications we report the genotype, the inheritance and the clinical features of six females with interstitial duplications of Xp22.31 ranging in size from 1,48 Mb to 1.76 Mb found by array-CGH analysis. Five of these individuals presented intellectual disability, whereas the genomic rearrangement was an incidental finding in the sixth individual.
The duplicated X chromosome was inherited in all the five patients. The inheritance was mainly paternal and all parents were clinically unaffected. Although the Xp22.31 triplication has been suggested to be more penetrant than the corresponding duplication, we found four copy of this region in one patient and three copy in her unaffected father.
In contrast with the more recent reports that increased the confidence about the pathogenicity of the Xp22.31 microduplication, our findings do not allow to confirm its association with a disease phenotype and cannot exclude the possibility that this is a rare population variant.

PS08.73
Recurrent Xq25 Microduplication: pathogenic or benign variant? A report of 11 cases from a French cohort

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Array Comparative Genomic Hybridization (CGH array), the first-tier diagnosis test for individuals with intellectual disability (ID), allows to describe numerous new microdeletion and microduplication syndromes. However, it is sometimes difficult to determine whether new Copy Number Variations (CNVs) are pathogenic or are benign variants because they are not well listed in the databases, their frequency in the general population is unknown and, most of the time, associated clinical data are missing.
Xq25 microduplication was first described by Bonnet et al. (2009) and Philippe et al. (2012) who reported a familial case and two sporadic cases respectively. These authors pointed out that the principal gene involved in this duplication was GRIA3. More recently, two publications by di Benedetto et al. (2014) and Yingjun et al. (2014) showed that the critical region involved the XIAP and STAG2 loci.
We report 11 individuals with Xq25 duplication diagnosed in the ACPA (Analyse Chromosomique sur Puce à ADN) network. This cohort consisted of 9 males and 2 females. The 11 duplications ranged from 180 to 965 kb. All these small duplications involved STAG2 and/or XIAP but only one duplication involved GRIA3. There was no minimum duplicated critical region shared by all these duplications. Ten individuals had mild to severe mental impairment but one individual was normally intelligent. In addition, the 11 individuals did not share any recognizable dysmorphic features. In conclusion, the findings in our case series do not provide evidence for the pathogenicity of the Xq25 microduplication.

PS09.001
Phenotypic expression of 15q11.2 microdeletion syndrome

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The 15q11.2 BP1-BP2 microdeletion contains TUBGCP5, CYFIP1, NIPA2 and NIPA1 genes implicated in neurodevelopment and usually presents with autism spectrum disorder, attention deficit hyperactivity disorder, developmental delay, hypotonia, ataxia, dyspraxia, seizures, psychiatric imbalances and mild dysmorphic features while athrogryposis is described in one case so far. Arthrogryposis includes various different conditions leading to reduced movement and joint contractures. We present the clinical and molecular findings of 2 affected male siblings (4 and 1 years old respectively) with 15q11.2 microdeletion syndrome who were initially referred to the Department of Medical Genetics because of arthrogryposis and mild psychomotor delay. They were both born at full-term gestation to healthy unrelated parents. The oldest sibling presented arthrogryposis of the hands at the age of 2,5 years old and later extended to the knee and elbows, while the younger sibling developed arthrogryposis at the age of 10 days. Their neurologic examination didn't disclose any abnormality and their muscular strength and tone appeared normal. High resolution 4X180K Agilent arrays (> 236.000 probes, average resolution of 8.9 Kb) revealed that they both carried 15q11.2 microdeletion (of approximately 400Kb), containing the TUBGCP5, CYFIP1, NIPA2 and NIPA1 genes. This is the second report of arthrogryposis presenting with 15q11.2 microdeletion syndrome, thus extending the previously described phenotypic spectrum of developmental delay- autism.

PM09.002
Molecular characterization of a de novo chromosomal rearrangement and in utero interference define ENO1 as a candidate gene for 1p36 polymicrogyria.

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While chromosome 1p36 deletion syndrome is one of the most common terminal subtelomeric microdeletion syndrome, 1p36 microduplications are rare events. We identified the smallest case of de novo 1p36 duplication reported to date using high resolution arrayCGH. The patient has intellectual disability, microcephaly, epilepsy and perisylvian polymicrogyria (PMG). PMG has frequently been observed in patients with 1p36 deletion syndrome. The duplicated segment in our patient is intrachromosomal and contains two genes : ENO1 and RERE, both disrupted by the rearrangement. Expression analysis of these two genes revealed a reduced expression in the patient cells, mimicking haploinsufficiency. We performed in situ hybridization during mouse development to study the developmental expression profile of these two genes. We also studied the consequences of their inactivation in vivo using in utero electroporation of shRNAs. These experiments allowed us to define the enolase 1 (ENO1) gene as the most likely candidate to explain the brain malformation phenotype of the studied patient and make it a good candidate for the malformations of the cerebral cortex observed in patients with 1p36 monosomy.

PS09.003
Genetic characterization of blood spot-derived DNA from 209 individuals known to carry 22q11Del reveals a new nested deletion

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Dried blood spot (DBS) samples from newborn babies are collected world-

wide to screen for diseases. In Denmark, blood spots have been stored since 1981 and combined with the well-characterized clinical information from the Danish registers, DBS is a valuable research opportunity for genetic population-based studies. The aim of this study is to evaluate the quality of copy number variants(CNV) in DNA derived from blood spots as well as to determine phenotypic risk with the known 22q11Del. DNA from 209 individuals identified from the Danish cytogenetic registry as carriers of a 22q11Del was extracted from DBS. Since blood spots contain limited amount of DNA, the DNA extraction step was carried out in parallel with and without the whole-genome amplification (WGA) step. The samples were genotyped with the Illumina Psych Array and PennCNV was applied for CNV prediction. Compared to unamplified genomic DNA, WGA DNA severely increases the CNV false positive rate. The true positive rate was not affected for the different versions of 22q11Del. Among the 209 individuals, 170 carried the common 3MB deletion, 15 the short 1.5MB deletion, 5 the atypical nested ~1MB deletion, 2 the atypical distal deletion and 4 carried a novel atypical deletion not previously described. The new deletion spans 6 genes and resides in the beginning of the common 22q11Del. This novel 22q11Del showed a similar psychiatric and somatic phenotype as the common deletion. The discovery of a novel 22q11Del that does not intersect with the well-studied candidate genes (TBX and COMT) underline the complexity of 22q11Del and the association to somatic and psychiatric diagnosis.

PM09.004

47, XXY (Klinefelter syndrome): Positive Effects of Early Hormonal Treatment (EHT) in Infancy and Hormonal Booster Treatment (HBT) between 5 and 8 years on Neurodevelopmental and Neurobehavioral Outcome.

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Background and Aims: 47, XXY occurs one in 500 live births and may result in complex neurodevelopmental and cognitive dysfunction. Three recent publications have demonstrated positive effects of Early Hormonal Treatment (EHT) during infancy.
Methods: 42 males prenatally diagnosed with XXY participated in comprehensive neurdevelopmental assesments. This retrospective study had three treatment groups: EHT with HBT, HBT-Only, and untreated. Data was analyzed one year before and one year after treatment. For the untreated group we used data points within 12 months of the treatment groups.
Results: The EHT+HBT group showed significantly better perfomance than the HBT-Only group on Fine Motor Control(p=.024) and PSI(p=.004). The EHT+HBT group also showed better performance than the untreated group on motor tests; Fine Motor(p=.017), Running Speed(p=.031), Strength(p=.032), the Motor Composite (p=.023), as well as on the WISC-VCI(p=.019), PRI(p=.021), and FSIQ(p=.004). The HBT-Only group performed significantly better than the untreated group on the VCI(p=.020) and PIQ(p=.010). There was a significant drop in PIQ in the untreated group over time (p=.033), while the treated groups did not decline.
Conclusions: Multiple positive effects of Testosterone replacement were observed in boys with 47, XXY, supporting the need for early identification and treatment of these boys. The significant results were observed in the EHT+HBT group. The untreated group showed a decline in PIQ over time. This suggests that, without treatment, some cognitive functioning may decline in young boys with 47,XXY. Androgen replacement prior to puberty may be important not only to improving cognitive function, but to preserving existing function in prepubertal males with XXY.

PS09.005

Severe psychiatric phenotype in a child with 47, XYY and multiple rearrangements detected in array CGH

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Co-occurrence of 47,XYY syndrome and other molecular rearrangements in the same individual is extremely rare. We report a boy referred for genetic evaluation at the age of 9 years old when he was admitted to the psychiatric unit of the Children's Hospital due to extended aggressive behaviour, which attributed to a severe psychiatric phenotype. He had no peculiar phenotypic findings, except for a unilateral iris coloboma. The proband's family and medical history were not indicative of any abnormality.

Cytogenetic analysis revealed a 47,XXY karyotype*, while the high resolution array based comparative genomic hybridization (aCGH) identified important rearrangements shown below:

Chromosomal region/ size	Position of 1st aberrant probe	Position of last aberrant probe	*Important Genes in aberrant region*	comments
DUP 7q11.23; 882.9Kb	74,144,422	75,027,348	*GTF2I, NCF1, GTF2IRD2, STAG3L2, PMS2P5, GATSL1, WBSCR16, GTF2IRD2B, NCF1C, LOC100093631, GTF2IP1, GATSL2, SPDYE8P, PMS2L2, STAG3L1, TRIM74, TRIM73*	Microduplication in the Williams syndrome region
DEL 11p15.5; 1.6Mb	383,89	2,014,937	*DRD4*	ADHD**
DEL 20q13.33; 907.3Kb	61,632,196	62,539,530	*HAR1B, HAR1A, CHRNA4, KCNQ2*	Psychotic behavior, ASD**, epilepsy

*Aberrations detected with aCGH due to 47XXY karyotype: DUP Xp22.33, 2.7Mb; DUP Xq21.31 - q21.32, 3.9Mb; DUP Yp11.32 - p11.2, 9.96Mb; DUP Yq11.21 - q12, 15.1Mb
ADHD ** Autism Deficit Hyperactivity Disorder; ASD: Autism Spectrum Disorder
This case underlines the utility of aCGH in addition to the routine cytogenetic analysis in cases with severe psychiatric phenotype for accurate diagnosis and complete genetic counselling.

PM09.006

Genetic overlap between Intellectual Disability and Attention-Deficit/ Hyperactivity Disorder

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Attention-Deficit/Hyperactivity Disorder (ADHD) is a common neuropsychiatric disorder with a complex genetic background. Intellectual Disability (ID) and ADHD are often co-morbid, suggesting genetic overlap. We investigated whether genes, affected by rare genetic variations in patients with ID, also contribute to ADHD risk. Common single nucleotide polymorphisms (SNPs; MAF>= 1%) in 384 autosomal ID-related genes selected from the 'Intellectual Disability Gene Panel' (downloaded from https://www.radboudumc.nl/Informatievoorverwijzers/Genoomdiagnostiek/Documents/ngs-intellectual_disability_panel_181213.pdf) were tested for association with ADHD risk, on gene-wide and gene-set level, using ADHD meta-analytic data from of the Psychiatric Genomic Consortium (PGC; N=5,621 cases and 13,589 controls) using KGG v3.5. The ID gene-set was significantly associated with ADHD in the PGC ADHD meta-analysis ($P_{gene\text{-}set}$=2.19E-3). Further analysis showed that the findings were due to the inattention symptom domain ($P_{gene\text{-}set}$=5.00E-3) rather than hyperactivity/impulsivity in ADHD ($P_{gene\text{-}set}$=0.203). The *MEF2C* gene showed gene-wide association (corrected for multiple testing) with ADHD risk (P_{MEFC2}=3.92E-5). Two SNPs within the *MEF2C* locus were assessed on neuroanatomy using voxel-based morphometry (VBM) in the Brain Imaging Genetics cohort (BIG; N>1,300) and one SNP showed bilateral association with gray matter volume in the lateral occipital cortex. This study demonstrates the genetic overlap between ID and ADHD, showing that common SNPs in genes, known to be affected by rare genetic variations in ID patients, contribute to ADHD risk. We also identified *MEF2C* as a novel candidate gene for ADHD.
Grants were received from Netherlands Organization for Scientific Research (NWO Brain & Cognition Excellence Program (433-09-229)), Vici grant to B.F. (016-130-669), Netherlands Brain Foundation (grant 15F07[2]27), BBMRI-NL (CP2010-33), and European Community's Seventh Framework Programme (FP7/2007-2013; 602805).

PS09.007

Association study of DISC1 and COMT polymorphisms in Bulgarian patients with Alzheimer disease.

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Background: Alzheimer disease (AD) is the most common neurodegenerative disease characterized by dementia, cognitive impairment, memory and personality disorders resulting in progressive degeneration of the brain neurons. DISC1 (disrupted-in-schizophrenia-1) and COMT (catechol-O-methyltransferase) are associated with the pathogenesis of neurocognitive impairment. The Val158Met (rs4680, G>A) polymorphism in COMT and Ser736Cys (rs821616, T>A) in DISC1 genes have shown association with various neuropsychiatric and neurodegenerative diseases. Several studies implicated them in the cognitive impairment and different neuropsychiatric features.
Materials and methods: In this study 218 patients with AD and 125 healthy controls, matched to the patients by age, gender and ethnicity (HC) were included. Genotypes were determined using TaqMan assay (Applied Biosystems). Statistical analysis was done using PLINK .
Results and discussion: The Val158Met and Ser736Cys polymorphisms did not show significant difference in allele frequency distribution between the AD and HC group. The common Val158Met alleles of COMT were with almost equal allele frequencies (p=0.9, OR= 1.02). Ser736 variant of DISC1 was more frequent among AD patients, but did not reach significant association (p=0.08, OR=1.37). When a subgroup analysis depending on the age of onset was done, the Ser736 variant of DISC1 showed tendency of association (p=0.055, OR=1.51)
Conclusions: Our findings did not show overall association with the studied COMT and DISC1 SNPs in Bulgarian patients with AD, probably due to the limited sample size. Further studies in enlarged sample and with cognitive measures are needed to elucidate their role.
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PM09.008

Analysis of allelic variants in endothelial function and cell death related genes in Spanish patients with vascular dementia and Alzheimer's disease

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INTRODUCTION: The two most common causes of dementia are Alzheimer's disease (AD) and vascular dementia (VD). The onset of dementia may be determined by genetic and environmental factors. Single nucleotide polymorphisms (SNPs) of genes encoding VEGF-A and its receptor, VEGFR-2, eNOS or TP53 might modulate the risk of these global health problems.
OBJECTIVES: We have analyzed the relationship of the following SNPs: -460C>T and -2578A>C of VEGFA, -604A>G of VEGFR2, +894G>T of eNOS and Arg72Pro of TP53 as well as sociodemographic and clinical risk factors VD develop (and its subtypes) and EA.
We have analyzed 150 patients with VD (corticalVD=74; subcortical VD=76), 147 patients with AD and 150 controls >70 years that there was no evidence of clinical or psychometric cognitive impairment. Genotyping was accomplished by DNA digestion with restriction nucleases (PCR-RFLP) and PCR with Taqman® probes (qRT-PCR).
RESULTS: Our results showed that VEGFA alleles 460C-2578A (OR:0.625, p=0.042) and eNOS allele +894G (OR:0.606, p=0.037) were associated with lower risk of EA in patients older than 80 years. The VEGFR2 allele -604A (OR:0.555, P=0.004) was associated with a lower risk of subcortical VD. The Pro72 allele of TP53 gene (OR:0.500, p=0.001) was associated with a lower risk of AD while the Arg72 allele (OR:1.768, p=0.026) was associated with an increased risk of cortical VD.
CONCLUSIONS: The results of our study suggest that polymorphisms associated with endothelial function and cell death may modulate the susceptibility to the risk of different subtypes of dementia.
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PS09.009

Angelman syndrome due to a maternally inherited intragenic UBE3A deletion in a family of Chinese origin

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Angelman syndrome is characterised by global developmental delay, lack of speech, seizures, a characteristic behavioral profile with a happy demeanor, microcephaly, ataxia etc. More than 2/3 of cases are due to a 5 Mb interstitial deletion of the imprinted region 15q11.2-q13, which is usually de novo. The rest are associated with point mutations in UBE3A gene, imprinting defects and paternal uniparental disomy. Small intragenic UBE3A deletions have been rarely described. They are usually maternally inherited, increasing the recurrence risk to 50%, and may be missed by conventional testing (methylation studies and UBE3A gene sequencing). Herein a boy with Angelman syndrome due to an 11.7 kb intragenic deletion is described. The deletion was identified by array CGH and was subsequently detected in his affected first cousin and unaffected maternal grandfather, mother and aunt, confirming the silencing of the paternal allele. The patient had global developmental delay, speech impairment, a happy demeanor, microcephaly and an abnormal EEG, but no seizures by the age of 4 years. The family was given a recurrence risk of 50% and prenatal testing was offered. Delineation of the underlying genetic mechanism is of utmost importance for reasons of genetic counselling, as well as appropriate management and prognosis. Alternative techniques, such as array CGH and MLPA, are necessary when conventional testing for AS has failed to identify the underlying genetic mechanism.

PM09.010

Genetic diagnosis of ataxia using NGS

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Hereditary Cerebellar Ataxia (HCA) is a slowly progressive disorder characterized by the presence of abnormal and uncoordinated movements that mainly affect walking, speech and eye movement. Its diagnosis is not straightforward, mainly due to the presence of nonspecific overlapping clinical symptoms and of genetic heterogeneity. A global approach using massive technologies such as NGS could be of great help to improve the diagnosis.
We present a dataset of 28 ataxic patients studied in our laboratory using a NGS targeted re-sequencing panel that included genes associated with Ataxia [between 1-30 genes]. Coding exons and splice-site regions of the genes associated with each pathology were analyzed. Enrichment and sequencing were carried out using SureSelect Enrichment System (Agilent) and SOLiD 5500/MiSeq(Life Technologies/Illumina). Mean depth was established at 200x.
In 6 out of 28 samples (21%), a genetic cause that justifies the clinical diagnosis was found. In 12 out of 28 patients the results were inconclusive. In these samples, 41 variants (confirmed by Sanger sequencing) were identified as variants of uncertain significance (VUS). According to their effect, missense (21), synonymous (13), in frame deletion (2), splice-site (5) mutations were found. In 10 out of 28 samples no variants (pathogenic/UV) were identified.
Based in our experience, we propose that:
• NGS is an effective approach for the diagnosis of Ataxias.
• Cosegregation studies and functional analysis are needed to better understand the effect of VUS and could aid in the diagnosis of a significant number of patients.

PS09.011

Diagnosing ataxia using a Next-Generation-Sequencing gene panel

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Introduction: The exact use of escalated genetic testing in a heterogeneous group of ataxia patients is unknown. Especially patients with an onset beyond the age of 40 may be underdiagnosed. This is particularly relevant in the light of treatable metabolic defects but also genetic counselling and clinical work-up require a correct diagnosis.Materials and Methods: 139 patients were sequenced using a Haloplex (Agilent) enrichment of 61 ataxia genes, 40 metabolic genes associated with ataxia and 17 genes causing mitochondrial disorders on a MiSeq instrument (Illumina, 150 bp paired-end sequen-

cing).Results: 9% were immediately diagnosed (3x SCAR8, 2x EA2, 2xSCA14, SCAR10, SPG7, NPC, SCA28, GM1 gangliosidosis, APBD) (6 with onset before, 7 after 40 years, 2 of which had a negative family history). Another 12% of cases were probably solved unless the segregation (not completed) yields controversial results (13 with onset before, 3 after 40 years; negative family history in 1). In 39% we found either heterozygous mutations or likely pathogenic variants in recessive ataxia-related genes or candidate variants in dominant genes facilitating a targeted diagnostic work-up for the referring clinicians (25 with onset before, 29 after 40 years, 14 of which had a negative family history). Conclusions: NGS is powerful in diagnosing hereditary ataxias but also provides a large number of variants requiring careful clinical evaluation. As our results indicate it is possible to diagnose even patients with a late onset or negative family history for mutations in ataxia-related genes.<br

PM09.012

The National Paediatric Ataxia-Telangiectasia (A-T) Clinic in England

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A-T is an early childhood progressive multisystem neurodegenerative disorder characterised by cerebellar ataxia, immunodeficiency, lung disease and a high risk of malignancy. Median survival is estimated at 25 years, but severity is variable and management is improving the quality of life and survival. A-T is recessively inherited and caused by mutations in the ATM gene on chromosome 11q.26. A-T is rare; in the UK and Ireland there are approximately 170 cases.
England has a nationally-commissioned Paediatric A-T Clinic based in Nottingham and sees children from the UK and overseas. There are two fundamental aims, to achieve an accurate diagnosis and to manage the patient holistically, addressing every aspect of the condition. The Clinical Team comprises over 20 specialists and collaborates with the A-T Society support group. Clinic is held 6 times a year and sees 6 children per clinic over 2 days. Generally, patients are reviewed every 2 years with ongoing contact with their local Paediatric Teams. We have recently published a guidance document on diagnosis and clinical care for children with A-T. Involvement in research and family education are integral parts of this Service.
The poster will describe the patient pathway, specific medical interventions undertaken in the clinic and/or arranged through local services as well as clinical outcomes and implications for management. Examples are the underlying ATM mutations, the nutritional status of patients, the prevalence of skin disease and scoliosis and the results of respiratory, immunological and other investigations undertaken.
We believe that this Clinic provides a paradigm for the optimal management of rare progressive multi-systemic genetic disorders.

PS09.013

Exome-wide association analysis of attention hyperactivity disorder in a genetically isolated population

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Attention deficit hyperactivity disorder (ADHD) is highly heritable psychiatric disorder with a heritability estimates ranging from 60 to 90 %. To identify rare variants associated to ADHD, we performed exome-sequencing based association study in a genetically isolated population in the Netherlands. The study included 554 individuals whose exomes were sequenced and who were assessed for inattention, hyperactivity and ADHD index. All single nucleotide (SNVs) variants were tested for association applying additive linear modeling adjusting for age, sex and relatedness among the samples. Moreover Sequence Kernel Association Test (SKAT) was used to test the joint effect of multiple variants within the gene/region. Significant association of two rare SNVs (MAF = 0.27 %, β = 12.70, p-value = 9.36E-08) mapped to intronic part of multiple C2 domains, transmembrane 1 (MCTP1) gene on chromosome 5 was observed with inattention. Gene-based analysis revealed suggestive association of CEACAM19 (p-value = 1.91E-05) and SCYL2 (p-value = 1.35E-05) to inattention and hyperactivity, respectively. To conclude, present study identifies exome-wide association between ASD and two SNVs within MCTP1 gene, which play important role in Ca2+ signalling at membrane. However, further studies are needed to confirm these findings.

PM09.014

CNVs in the group of the patients with autistic spectrum disorders detected by MLPA and SNParray.

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Autistic spectrum disorders (ASD) are characterized by simultaneous deficits in 3 domains of behavior: reciprocal social interaction, communication and stereotyped and restricted behaviors. Submicroscopic CNVs can have a causal or susceptibility-related role in heritability ASD.
Objective: We performed MLPA and SNP array study focused on detection of CNVs in the group of patients with ASD.
Method: Samples of DNA were tested by MLPA (SALSA MLPA P343 Autism 1, Microdeletions - P297, P245, Subtelomeres -P070, P036 and P106 MRX) and SNP array.
Results: In the group of the children with ASD (42) three likely pathogenic CNVs were discovered. Microduplication 16p11.2 (576 kb) inherited from father, microduplication 1q21.1q21.2 (1,3 Mb) de novo, unbalanced translocation with microdeletion 20q13.33 (1,58 Mb) and microduplication 22q13.33 (app. 190 kb - covering SHANK3) de novo. In another four cases, the inherited variants of unknown significance involving genes: NRXN1, SNRPN, MAPK3, and CD160 were detected.
The microdeletion 16p11.2 (611 kb) was incidentally found in the female patient with Mayer-Rokitansky -Kuster-Hauser syndrome (MRKHS) without autistic features. The loss of 1.3 Mb in 15q13.2q13.3 was also discovered by MLPA 343 Autism1 in the patient suffered from intellectual disability but without ASD.
Conclusion: SALSA MLPA probemix P343 Autism1 has proved to be efficient for detection CNVs in the group of children with ASD. However, the simultaneous tests with another probemixes (P297, Human Telomere 3 and 5 and so on) or simultaneous test with SNP or CGH array is recommended especially when intellectual insufficiency in the patient is present.

PS09.015

Whole exome sequencing identified a novel missense variant (c.425C>G or p.P142R) of the EN2 gene in two unrelated Thai patients with autism spectrum disorder

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Autism spectrum disorder (ASD) is a group of neurodevelopmental disorders with largely unknown genetic defects. Recently, whole exome sequencing (WES) has been found to be a powerful technique for identifying new variants which may help to depict the complex genetics of ASD. Nine unrelated Thai patients with ASD and no pathogenic CNV were selected for WES study using the SureSelect Human All Exon V4 kit on the Illumina Hiseq2000. To identify potential pathogenic variants in candidate genes of ASD, the TruSight Autism gene list (101 genes) and SFARI AutDB database (667 genes) were used for reference databases. Interestingly, a novel heterozygous missense variant (c.425C>G or p.P142R) in exon 1 of the Engrailed 2 (EN2) gene, a homeobox transcription factor involved in brain development, was identified in two patients. In the first family, the G allele was also found in the patient's older brother with intellectual disability and his normal mother. In the second family, the G allele was transmitted from the patient's normal father, but it was not found in a male cousin with ASD. In addition, the variant was absent in the SNP database, the Exome Variant Server (EVS), the 1000 Genome Project, 100 Thai patients with ASD and 240 normal Thai controls suggesting that the new variant is very rare and has never been reported. Although this variant is predicted by the PolyPhen-2 to be possibly damaging to the EN2 protein function, EN2 protein function studies of the p.P142R are required to determine molecular basis of ASD pathogenesis.

PM09.016

The autism-associated long noncoding RNA MSNP1AS regulates a network of genes involved in neuronal process stability.

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From genome-wide association studies (GWAS), a novel gene was discovered that has a highly significant association with autism spectrum disorder (ASD). The gene is a long noncoding RNA (lncRNA) designated *MSNP1AS* (moesin pseudogene 1, antisense). Expression of *MSNP1AS* was increased in the cerebral cortex of individuals with ASD and individuals with the ASD-associated genetic marker. Overexpression of *MSNP1AS* in human neuronal cells caused decreased expression of moesin (*MSN*) mRNA and moesin protein, which is involved in neuronal process stability and immune response. These data indicate one aspect of the potential contribution of increased *MSNP1AS* expression in ASD. However, there are likely to be additional transcriptomic impacts of this lncRNA. To determine the effects of altered *MSNP1AS* expression on the neuronal transcriptome, we transfected human neuronal progenitor cells with constructs that overexpressed *MSNP1AS* or transcriptionally silenced *MSNP1AS*. RNA-Seq analysis indicated altered expression of multiple genes that contribute to altered neuronal process stability and immune response, including *MSN*. However, our data indicate several genes that are impacted by *MSNP1AS* dysregulation more significantly than *MSN*, suggesting a network of genes that contribute to ASD risk. Ongoing experiments seek to define the role of the *MSNP1AS* gene network in neuronal process stability.

PS09.017

Expression profile of circulating miRNAs in Autism Spectrum Disorder (ASD)

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Introduction: ASD is a genetically complex disorder, highly heterogeneous and with unclear etiology. In this study we addressed the possible contribution of epigenetic factors to ASD etiology, focusing on deregulated MicroRNAs (miRNAs) expression. miRNAs are small noncoding RNA molecules that negatively regulate gene expression. miRNAs are thought to be released from pathological tissues to plasma in illness situations, and the strong correlation between circulating and tissue miRNAs indicates that plasma miRNAs may be useful disease biomarkers.
Methods: miRNA plasma profiles were obtained for patients with ASD and other neurodevelopmental disabilities (NDD), essentially disruptive behavior, language delay and psychomotor delay. Patients were all male, with a mean developmental coefficient of 92±18, and no obvious dysmorphisms. Exiqon human miRNome PCR panels (testing 752 miRNAs) were used for miRNA profiling.
Results: We identified 50 miRNAs differentially expressed in ASD vs NDD. Notably, of the 10 miRNAs with higher fold-change (increased 2.5-3.3X), 8 miRNAs had a target gene in common, MMP16. This gene is expressed at high levels in the brain, and a balanced de novo translocation in a ASD patient involving MMP16 has previously been described.
Conclusions: This is the first study of circulating miRNA profiles comparing patients with ASD and other NDDs. The preliminary results suggest that miRNA profilling may eventually improve differential diagnosis. The results also implicate novel pathological pathways in ASD, namely involving metaloproteinases. These are crucial molecules for the integrity of the extracellular matrix and consequently for cell migration and regeneration, processes thought to be involved in ASD.

PM09.018

Autistic variome: gene hunting through SNP-microarray genome analysis and bioinformatics

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Despite of significant advances in bioinformatics and genomics, analyses of generalized data on copy number variation (CNV) or variome aimed at gene hunting still represent a major challenge. Here, we have used an approach towards identifying genes implicated in autism pathogenesis through evaluation of autistic variome addressed by microarray genome analysis with an original bioinformatic technology. To identify the spectrum of CNV, a high-resolution SNP-array genome scan (Affymetrix platform) was used. Bioinformatics analysis has represented a complex evaluation of CNV by simulations of transcriptional, interactomic and metabolomic activity of candidate genes using available genome, transcriptome and proteome databases (Iourov et al., 2014). Pathogenic CNV were found in 38 children with autism from the Russian cohort (n=212). Six chromosomal rearrangements were detected: deletions at 6p11.2 (PRISM2), 9q21.13 (GDA, ZFAND5, TMC1), 8p23.3p23.1 (46 genes), Xp22.12 (RPS6KA3, CNKSR2) and Yq11.223q11.23 duplications. Furthermore, bioinformatic analysis of CNV and single-gene deletions/duplications has defined following autism candidate genes: CBARA1, KRT83, RBFOX1, NDP, ATP6V1E1, CNKSR2, ATP1A2, FBXO21, ACSL3, ATP2B3, IMPA1, CNTNAP4. The latter allowed speculations on potential autistic molecular pathways involving genome instability, programmed cell death and axon guidance. Successful delineation of a panel of candidate genes suggests generalized variome analyses as a valuable source for gene hunting. These data supports the idea that additional bioinformatic technologies are able to give further insights into disease pathogenesis. Thus, a disease variome determined by SNP-array genome scan and addressed an original bioinformatic technology appears to be an issue for genomic studies. Supported by Russian Scientific Fund (Grant #14-35-00060).

PS09.019

Identification of a rare deletion encompassing ELMOD3 and CAPG in two siblings with Autism Spectrum Disorder

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Autism Spectrum Disorder (ASD) is a severe neurodevelopmental condition with highly complex genetic predisposition. Recent discoveries have highlighted the importance of both sequence and structural rare variations in ASD susceptibility. In particular, focusing on rare copy number variants (CNVs) has already been highly productive in uncovering an increasing number of specific genes and chromosomal intervals conferring risk to ASD.
During a genome-wide CNV scan using a multi-algorithm approach on 9 multiplex ASD families from Sardinia, we identified a rare genic deletion of ~37 Kb, transmitted from the unaffected mother to both affected siblings. This deletion includes the entire *CAPG* gene and the last coding exons of *ELMOD3*. *CAPG* encodes a member of the gelsolin/villin family of actin-regulatory proteins, that might be involved in control of dendritic spine shape. ELMOD3 belongs to the engulfment and cell motility (ELMO) family, with recently proposed functional links to sound perception and actin cytoskeleton.
No deletions involving this genomic region were found in 4768 controls from published high resolution SNP-array data, while other two deletions encompassing *CAPG* and *ELMOD3* have been reported in two subjects with ASD, suggesting that *CAPG* and/or *ELMOD3* haploinsufficiency may have clinical relevance to ASD. In order to test this hypothesis together with a recessive model of inheritance, we first excluded that this deletion is a common polymorphism in Sardinia, then we performed expression and mutation analysis of both genes in the discovery pedigree, and a further clinical evaluation of all family members.
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PM09.020

Exome sequencing of two large Cuban families densely affected with bipolar disorder

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Bipolar disorder (BD) is a major psychiatric disorder affecting more than 1% of the world's population. The disease is characterized by recurrent manic and depressive episodes and shows a high heritability of about 70%. To date several genes contributing to BD susceptibility have been identified. However, the disease driving pathways and regulatory networks remain largely unknown. As the cumulative impact of common alleles with small effect may only explain around 38% of the phenotypic variance for BD (Lee et al., 2011), rare variants of high penetrance have been suggested to contribute to BD risk.

In the present study we investigated the role of rare nonsynonymous variants in two large Cuban families. The exceptional lifetime prevalence of BD in these pedigrees makes them promising candidates for identifying genetic risk variants of Mendelian-like effects. For this purpose we selected 16 affected individuals from both families and performed exome sequencing on the HiSeq platform. In addition, all family members with available DNA (n=34) were genotyped on the Illumina Psych Chip to create polygenic risk-score analyses in order to evaluate the contribution of common variants in the two pedigrees.

Exome sequencing has recently been completed. Preliminary results of the first family revealed 10 missense variants and two frameshift variants shared among all investigated individuals (n=4). Affected genes include ADCY3, a gene that has been suggested to play a role in major depressive disorder, and SEMA4C required for brain development. Further evaluation and interpretation of these results is currently underway and will be presented.

PS09.021

Delineation of the mutational spectrum in two susceptibility genes for bipolar disorder, neurocan (NCAN) and adenylate cyclase 2 (ADCY2)

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Bipolar Disorder (BD) is a common, genetically complex neuropsychiatric disorder with a significant impact on the global burden of disease. Patients suffer from recurrent episodes of strongly elevated (mania) and depressed mood. The life-time prevalence ranges between 0.5-1.5%. Heritability for BD estimates between 60-80%.

We have recently published two large Genome Wide Association Studies (GWAS) that provide strong evidence for an involvement of genetic variants in neurocan (NCAN, Cichon et al. 2011) and adenylate cyclase 2 (ADCY2; Mühleisen et al. 2014) in BD. NCAN encodes a glycoprotein that plays a role in neuronal development. ADCY2 is a key enzyme in cAMP regulated GPCR signalling pathways.

For GWAS association signals, it is normally unclear whether they represent the functionally relevant variants or just proxies in linkage disequilibrium. Therefore, to uncover the whole mutational spectrum in NCAN and ADCY2 and to detect the putative functional variant underlying the GWAS association signal, we conducted a Next-Generation-Sequencing based re-sequencing in 960 German BD patients. We designed a custom amplicon panel using Illuminas TruSeq Custom Amplicon©-Kit and performed the sequencing on a MiSeq© System from Illumina©. Alignment and variant calling was done using the MiSeq reporter tool v2.4 and variant annotation according to HGVS was conducted in Illumina VariantStudio©. Three exons were separately sequenced using Sanger-Sequencing.

Our preliminary analysis with stringent quality criteria detected 178 unique variants, 78 of them being missense variants. We are currently focussing on the in silico functional characterization of the missense variants with the results being presented at the conference.

PM09.022

Enhancer variant enrichment analysis in bipolar disorder

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Bipolar disorder (BD) is a severe neuropsychiatric disorder and GWAS have robustly identified genetic variants implicated in disease susceptibility. However, the majority of loci are located in non-coding regions of the genome, thus requiring further functional annotation.

Enhancers are distal regulatory elements that control the activation of tissue- and cell-type specific gene expression. Large-scale identification of actively transcribed enhancers is performed by different techniques such as chromatin immunoprecipitation or cap analysis gene expression coupled with next-generation sequencing. The Fantom5 enhancer atlas (Andersson et al. 2014) represents a systematic resource for active enhancers in tissues including human brain. The integration of GWAS signals and these functional genomic regions provides novel opportunities to elucidate biological mechanisms underlying BD. Therefore, we investigated in this study whether brain related enhancers are enriched among top BD GWAS hits.

Results from the largest BP meta-analyses comprising a sample of 24,025 patients and controls (Mühleisen et al. 2014) provided the basis for this analysis. We mapped significant GWAS to brain enhancer regions defined by the Fantom5 study. Enrichment of associated SNPs in enhancers was assessed by permutation analysis comparing observed overlap for each enhancer to randomized enhancer regions.

An enrichment of GWAS hits was observed in neurons (p=0.015) but not in human brain enhancers suggesting a cell-type specific regulation. A detailed analysis of several brain regions is currently ongoing and will be presented. Overall, our results provide the first systematic integration of brain-related enhancers in the largest BP GWAS to date and subsequently enable the discovery of relevant biological processes suitable for further functional studies.

PS09.023

Identification of shared risk loci and pathways between bipolar disorder and schizophrenia

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Bipolar disorder (BD) is a highly heritable disorder of mood with a lifetime prevalence of about 1%. BD shows substantial clinical and genetic overlap with other psychiatric disorders, particularly with schizophrenia (SCZ). However, research has not yet clarified what particular genes form the basis of this etiological overlap.

For both disorders several susceptibility genes have been identified. In the case of SCZ, a meta-analysis (36000 patients, 113000 controls) of the Psychiatric Genomics Consortium (PGC) identified 128 independent genome-wide significant SNPs.

The aim of the present study was to investigate whether these 128 SCZ-associated SNPs also contribute to BD development. We conducted association testing in our large GWAS dataset of BD (9747 patients, 14278 controls, Mühleisen et al., 2014). In this dataset we combined our data with the BD GWAS results of the PGC (Sklar et al., 2011). As different reference panels were used for the imputation of the genotype data in both studies, we reimputed the summary statistics of the PGC BD GWAS using a method by Pasaniuc and colleagues (2014) and performed a meta-analysis using METAL. Overall, 107 SCZ-associated SNPs could be mapped to our reimputed data. Our analysis revealed that 42 of the 107 SNPs showed nominally significant p values, providing further evidence that SCZ-associated loci contribute to BD development (p<2.2x10-16). Pathway analysis (INRICH, Ingenuity pathway analysis) for all 42 shared SCZ-BD SNPs revealed a total of 27 nominally significant pathways including calcium and glutamate signaling.
Our results may provide clues for new approaches to treatment and prevention of BD and SCZ.

PM09.024

C9ORF72-related FTD/ALS: a study of expansion size, gene expression and DNA methylation

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Introduction: A hexanucleotide GGGGCC expansion in *C9ORF72* accounts for up to 40% of familial ALS and up to 25% of familial FTD (c9FTD/ALS). The pathogenic mechanism of this disease remains unknown. One possibility is that the expansion induces *C9ORF72* loss-of-function. In support of this, methylation of *C9ORF72* promoter DNA has been reported in association with decreased gene expression. However, the relationship between expansion size, gene expression, and promoter methylation remains unclear.
Materials and Methods: Fibroblasts from c9FTD/ALS patients were cultured and analysed for hexanucleotide repeat size by Southern blotting. *C9ORF72* gene expression was determined by qRT-PCR and digital droplet PCR. The degree of promoter DNA methylation was assayed by methyl-DNA immunoprecipitation with qPCR. The expression of wild-type mouse *C9ORF72* orthologue was also assayed by qRT-PCR across ages from birth to 1 year.
Results: *C9ORF72* expansion size in fibroblasts did not correlate with either gene expression or promoter methylation. Surprisingly, there was a positive correlation between degree of methylation and levels of *C9ORF72* expression. Of the three described *C9ORF72* mRNA transcripts, one predominates that uses a first exon downstream of the expansion. In mice, brain expression of the *C9ORF72* orthologue was found to be significantly higher in the neonatal period.
Conclusions: This study demonstrates a wide variability in *C9ORF72* gene expression and promoter DNA methylation among patient cell lines carrying pathogenic hexanucleotide expansions. The high levels of expression in neonatal mice suggests a role in neurological development.
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PS09.025

An audit of two years of diagnostic testing for the C9orf72 GGGGCC expansion in FTD/ALS

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The GGGGCC expansion in the intronic/promoter region of C9orf72 was first described in October 2011 and is the most common monogenic cause of Frontotemporal Dementia and/or Amyotrophic Lateral Sclerosis (FTD/ALS). Non-pathogenic alleles are typically 2-20 repeats, and pathogenic expansions typically several hundred to several thousand repeats in length, with considerable somatic mosaicism. The smallest pathogenic repeat size is currently unknown. We have offered a diagnostic service for the expansion at the National Hospital for Neurology and Neurosurgery since January 2013. We employ repeat-primed and sizing PCRs to detect expansions and to size alleles in the non-pathogenic range, respectively. Southern blotting is used to confirm and size expansions and to exclude false negatives in patients who are homozygous with no expansion by PCR. Of 242 reports issued up to February 2015, 240 were diagnostic, with 2 predictive. Of the diagnostic tests, 27 were positive (11.25%), this is concordant with the published frequency of the expansion in FTD/ALS. Among our positive results 24 had a typical expansion. We have also identified one apparent homozygote, one patient with an expansion of 90 repeats in blood which had expanded to the typical pathogenic range in brain tissue, and one patient with a repeat from 60 to several thousand repeats in blood DNA. These cases, and the genetic counselling challenges they present, exemplify the necessity of performing Southern blotting for C9orf72 diagnostic testing, without which the precise nature of the mutations would not have been identified.

PM09.026

A 5-year-old boy with progressive epileptic encephalopathy and severe developmental delay caused by novel compound heterozygous mutations in the CACNA1A gene

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Familial hemiplegic migraine type 1, episodic ataxia type 2, and spinocerebellar ataxia type 6 are autosomal-dominant neurological disorders associated with heterozygous mutations in the CACNA1A gene. Some patients may exhibit overlapping phenotypes, which combine various signs characteristic of these three conditions. The CACNA1A gene encodes the pore-forming α1 subunit of the neuronal voltage-gated calcium channel Cav2.1 mediating the action-potential-evoked neurotransmitter release in central nervous system.
In the index patient absent eye contact was noticed at the age of 2-months. Severe muscular hypotonia, arrested development and frequent epileptic seizures were observed later. At first MRI revealed white matter hypomyelinisation and thin corpus callosum, thereafter diffuse cerebellar and cerebral atrophy developed. At 5-years, he has profound developmental delay, muscular atrophy with rigidity, dysmorphic features and treatment resistant epilepsy. He is blind. Muscle biopsy showed lipid deposits in muscle fibers. His elder sister died at 5-years due to similar epileptic encephalopathy. Two elder sisters and both parents have mild intellectual disability. The mother has also ataxia and cerebellar atrophy.
Exome sequencing identified novel compound heterozygous mutations in the CACNA1A gene - a missense mutation c.4315T>A predicted pathogenic and a frameshift deletion c.472_478delGCCTTCC. Sanger sequencing confirmed both mutations in the index patient and his deceased sister. The mother and one elder sister carry c.4315T>A, and the father and the second elder sister carry c.472_478delGCCTTCC mutation.
This is the first description of a patient with a compound heterozygous mutation in the CACNA1A gene, which causes severe epileptic encephalopathy.
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PS09.027

Searching for a gene responsible for cerebellar ataxia and mild intellectual disability

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Introduction: Ataxia is defined as lack of control in voluntary movements. It may affect walking, speaking, and eye movements. It is a neurological condition that generally results from dysfunction of the cerebellum. It can be the sole symptom in a patient or can be present with other clinical features. We investigated a consanguineous family with syndromic ataxia.
Materials and Methods: Candidate disease loci were detected by linkage analysis using SNP genotype data. Exome sequencing was applied to search for variants at the loci.
Results: The affected sib had ataxia, intellectual disability, and aggressive behaviour. The maximum multipoint LOD score was 2.15. We identified homozygous nonsense mutation p.Glu39X in *FAM160B1* in the patient. The mutation truncates the protein by 95% and is not present in 302 control samples. The sequence of the first 736 of the total 765 amino acids is highly conserved between human and chimpanzee, with a difference of only 5. The function of the protein is yet unknown,unknown and it is shown to be expressed in many different tissues. The conserved domain has homology to retinoic acid induced proteins. One gene encoding such a protein Retinoic acid-induced 1 (RAI1) is associated with several neurological diseases. GeneMANIA suggests that FAM160B1 interacts with ATXN1, deficit of which is a cause of spinocerebellar ataxia.
Conclusion: We propose that *FAM160B1* is most likely the gene responsible for this novel syndrome. We will perform gene expression studies and screen other ataxia patients for mutations in the gene.
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PS09.029

Screening of CHCHD10 in a large French cohort with frontotemporal dementia and amyotrophic lateral sclerosis.

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Recently, we have identified a heterozygous mutation in the CHCHD10 gene in a large family with a late-onset complex phenotype including frontotemporal dementia (FTD), amyotrophic lateral sclerosis (ALS) and/or cerebellar ataxia, associated with mitochondrial myopathy and accumulation of multiple mtDNA deletions. CHCHD10 encodes a protein located in the mitochondrial intermembrane space and is likely involved in mitochondrial genome stability and maintenance of cristae junctions. Given the unawareness about the exact contribution of CHCHD10 in FTD-ALS spectrum, we evaluated, in this study, the frequency of CHCHD10 mutations in a large French cohort of 115 unrelated patients with FTD and FTD-ALS phenotypes, including 35 familial and 80 sporadic cases. We identified 2 heterozygous variants in 3 unrelated probands presenting FTD and ALS : the known p.Ser59Leu mutation in a familial case and the p.Pro34Ser variant in two sporadic cases. The phenotype of this probands is characterized by early and predominant bulbar symptoms. A short time ago, many studies confirmed the involvement of CHCHD10 in familial and sporadic ALS cases. However, this is the only study that demonstrates the implication of CHCHD10 in FTD-ALS spectrum. Although the frequency of mutations is low in this series (2.6%), our work suggests that CHCHD10 mutations should be searched particularly in presence of bulbar symptoms at onset, or evocative mitochondrial symptoms. The identification of CHCHD10 mutations in FTD-ALS spectrum opens a novel field to explore their pathogenesis and to understand the role of mitochondrial dysfunction. Further investigations in larger populations with different geographic origins are needed.

PM09.030

Functional studies of an LRSAM1 gene mutation causing Charcot-Marie-Tooth disease

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Introduction: Charcot-Marie-Tooth disease (CMT) is the most common inherited neuropathy of the peripheral nervous system affecting 1 in 2500 individuals. The LRSAM1 gene has recently been implicated in the CMT pathogenesis and very little is currently known about its role. LRSAM1 is an E3 ubiquitin ligase that has a significant role in endocytic and adhesion pathways in neuronal cells and mediates monoubiquitination of TSG101 at multiple sites. This study is focused on the functional analysis of the novel LRSAM1 c.2047-1G>A dominant splice-site point mutation. This mutation is located in the C-terminal RING finger domain of the encoded protein and leads to premature truncation (p.Ala683ProfsX3).
Materials and methods: Total RNA was extracted from available patient lymphoblastoid cell lines and the whole cDNA was synthesized. Initially, RNA levels of TSG101, the only known substrate of LRSAM1 E3 ubiquitin ligase activity, were investigated. Moreover, the RNA levels of E2 (ubiquitin) and additional molecules were examined. Selected molecules that possibly interact with LRSAM1 were detected using public protein-protein interaction databases.
Results: RNA levels of TSG101 and E2 were significantly reduced in patient compared to control levels. Furthermore, a statistically significant reduction of the levels of some of the selected molecules was observed. Western blot analysis to confirm the decrease of these molecules at the protein level is currently underway.
Conclusions: Our results provide more insight into the LRSAM1 pathways and molecular mechanisms, based on the alterations/disruptions caused by the LRSAM1 c.2047-1G>A mutation.
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PS09.031

Recurrent microduplication of chromosome 15q11.2 pinpoints risk genes for neurodevelopmental disorders

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Array-CGH analysis has shown that rare or common Copy Number Variants, both inherited and *de novo*, may significantly contribute to the etiology of neurodevelopmental disorders. A recurrent genomic aberrations associated with these phenotypes is the duplication of chromosome 15q11.2. Characterizing a cohort of 180 families using the Agilent 180K CGH array platform, we identified 4 patients carrying a 15q11.2 duplication involving the 4 genes TUBGCP5, CYFIP1, NIPA2 and NIPA1. Two were inherited from the healthy mother, one from an affected mother and one had an unknown parental origin. The duplication was confirmed by qPCR and a significant overexpression of the four duplicated genes was found using quantitative gene expression analysis. Two patients were diagnosed with Autism Spectrum Disorder, one with Developmental Delay and one is non-verbal and has a severe Intellectual Disability (the latter patient carries an additional pathogenic imbalance on the X chromosome). Strong evidence supports the role of this duplication in abnormal neurodevelopment. These four genes are all implicated in axonal growth, neuronal connectivity and morphology, especially CYFIP1 which regulates translation at the synapse by binding to FMRP. Phenotypically normal and mildly affected carriers complicate the phenotypic association and/or causality of this aberration. The explanation of this variability may be found in the reduced penetrance or altered gene dosage on a particular genetic background. This region likely represents a strong susceptibility region, as reported for other parts of the genome involved in neurodevelopmental disorders.

PM09.032

ZBTB20 mutations in corpus callosum anomalies

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Corpus callosum (CC) is the major brain commissure connecting the homologous areas of both hemispheres at the midline. CC malformations (CCM) are the most frequent brain malformations with an incidence of 1/4000 newborn often associated with chromosomal anomalies or mendelian syndromes with recessive and dominant inheritance. Recurrence is observed in 5%. Children with CCM have an uncertain neuro-developmental outcome. Therefore, counseling remains challenging prenatally.
On a cohort of 64 fetuses with CCM as autopsy finding, and 34 children with CCM and mental retardation, we used a targeted high throughput sequencing strategy, Here we report 4 novel ZBTB20 mutations, the recently identified causing gene for Primrose syndrome. We describe the clinical and or neuropathological and imaging data of one fetus and 3 patients. The p.Arg613Cys, p.Cys636Arg and p.Cys611Tyr (NM_001164342.1) mutations occur within the Cys2His2 zinc finger domains of ZBTB20. Cys2His2 zinc fingers consist of two cysteines and two histidines that co-ordinate a zinc ion, and an alpha-helix that makes sequence-specific contacts with DNA. We predict that these variants would destabilize the structure of their respective zinc fingers. The p.Val185Ile substitution falls within the BTB domain of ZBTB20 that is predicted to form homodimers and leads to an apparently milder phenotype .
These results suggest that a ZBTB20 mutation found in 4/98 individuals of our series is a frequent cause of CCM.

PS09.033

Next generation sequencing for cortical brain malformations: two years of experience

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Cortical brain malformations (rare disorders of proliferation, neuronal migration or cortical organization) have been associated with mutations in a rapidly growing number of genes. This complicates molecular diagnostics by Sanger sequencing. In order to establish a Next-generation sequencing (NGS) based work flow for routine DNA diagnostics, we developed a gene panel consisting of 103 relevant genes.
This workflow involved the design of enrichment arrays (Agilent Sure Select) for all genes, followed by Miseq sequencing (paired-end, 150bp, Illumina). BAM files are generated with an in-house analysis pipeline and variant calling is performed using the SeqNext software package (JSI).The average vertical coverage for exons is about 300; very few fragments fail to reach the minimal required vertical coverage of 30.
Furthermore, we were able to detect deletions up to 65 bp, insertions up to 29 bp and somatic mosaicism. Sanger-sequencing is used to confirm (candidate) mutations. DNA samples of 168 patients were tested and 6% of the cases could thus far be solved, including familial segregation analysis and confirmation of parentage, even in the absence of phenotypic information. However, most of the identified alterations are variants of unknown clinical relevance. Despite the use of in silico prediction programs, evaluation of the conservation among species they cannot be judged without additional clinical information and feedback from the referring physician.
In conclusion, the targeted NGS-panel for neuronal migration disorders is a powerful tool for DNA diagnostics. In order to increase the diagnostic yield of cortical brain malformations, close collaboration between laboratory and referring specialist is mandatory.

PM09.034
Long non-coding RNAs FENDRR, RNCR3 and MEG3 are aberrantly expressed in Creutzfeldt-Jakob disease

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Introduction: Non-coding RNAs, such as miRNAs and long non-coding RNAs (lncRNAs), are emerging as key regulators of cellular processes. Functional analyses of first lncRNAs revealed their significance in genome regulation and development. It is suggested lncRNAs *FENDRR*, *MEG3* and *RNCR3* are involved in brain and neurons development, and function. Regarding lncRNAs crucial role in various human diseases, our purpose was to investigate their possible involvement also in mechanisms of Creutzfeldt-Jakob (prion) disease and other neurodegenerative disorders.
Materials and Methods: We performed qPCR analyses to determine differential expression of three lncRNAs using autopsy brain tissue samples of cerebellum (C) and frontal lobe (FL) of 25 patients diagnosed with Creutzfeldt-Jakob disease (CJD), 20 non-CJD patients and 8 patients without neurological disease (WND). All results were compared to human brain reference RNA and statistically evaluated for significant expression differences.
Results: *MEG3* and *RNCR3* were strongly down-regulated in all sample groups, especially in CJD samples. *FENDRR* was up-regulated in all non-CJD and WND samples, and either down- or up-regulated in CJD samples. Statistical analyses showed significant over- or under-expression when we cross compared expression of CJD, non-CJD and WND patients. Moreover, significant expression differences were also observed between FL and C region within sample group.
Conclusions: Our findings support lncRNA's *FENDRR*, *MEG3* and *RNCR3* involvement in development of the prion disease and other neurodegenerative disorders, like Alzheimer's, and suggest they are necessary for normal brain function. Moreover, our results showed different spatial (cerebellum and frontal lobe) distribution that may contribute to the disease development.

PS09.035
Exome sequencing identifies SEC31A as a potenital candidate gene for schizophrenia

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Patients with schizophrenia (SCZ) suffer from a variety of symptoms, including delusions, hallucinations, disorganized speech and behavior. Even though studies have reported a reduced fecundity in patients with SCZ, the incidence rate of SCZ remains fairly stable. One hypothesis is that the mutations that are selected out of the gene pool due to strong negative selection are replaced by de novo mutations. Therefore, each gene identified to carry a de novo mutation is a potential SCZ candidate gene.
The exomes of 40 patients with a DSM-IV diagnosis of SCZ and their parents were analyzed. Exome DNA was captured from genomic DNA using NimbleGen in solution based capture and sequenced on an Illumina HiSeq 2000 sequencing instrument. The Varbank pipeline v.2.1 and interface were used for data analysis and filtering.
Subsequently, additional sources of genetic data were employed to gather more evidence for an involvement of the genes implicated: the genes were screened for the presence of CNVs using our existing genome-wide CNV dataset comprising of 1 637 patients and 1 627 controls. An association between common variants in these genes and SCZ was tested in the worldwide largest SCZ GWAS published by the PGC Schizophrenia Working Group analyzing more than 100 000 individuals.
So far, we have validated 28 different variants using Sanger Sequencing. Interestingly, our study is the third to report a de novo mutation in SEC31A. The combination of our exome sequncing and CNV data as well as the common variants will allow us to prioritize the variants identified regarding their relevance for the development of SCZ.

PM09.036
Clinical utility of gene panel testing: Case study using a 65-patient Dementia cohort

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The increasing availability and affordability of whole exome sequencing is challenging the current testing algorithms typically including detailed family history and clinical characteristics, predicting a decrease for the need of probabilistic prioritisation of genetic testing. To evaluate this prediction, we have assessed a cohort of 65 patients referred for frontotemporal dementia (FTD) and compared the diagnostic yield of the initial referrals for single gene tests vs. a 16-gene dementia panel test using our clinical exome sequencing pipeline. This 16-gene panel includes mostly autosomal dominant rare Mendelian early onset Alzheimer and FTD genes, that despite being clinically different show a degree of phenotypic overlap justifying the use of a panel.
Although this approach has the clear potential to increase the diagnostic rate, we also aimed to determine its usefulness vs. the burden of finding variants of unknown clinical significance (VUS). Analysis showed a diagnostic rate of 6/65 for the initial targeted referrals with 4/65 VUS. Subsequent interpretation of panel data increased this rate to 7/65 for published pathogenic variants and added 19 variants which we are currently assessing for pathogenicity, including 3 PSEN2 variants reported in the AD&FTD database. Preliminary results on this small FTD cohort indicate that despite the complexity of the interpretation of gene panel data, the increase in diagnostic rate together with value for money should encourage clinicians to refer for gene panels.

PS09.037
IL-6 and adolescent depression: A Mendelian randomisation approach

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Cross-sectional studies report an association between increased serum interleukin-6 (IL-6), an inflammatory marker, and depression. However, due to the nature of cross-sectional data, confounding and reverse causality cannot be ruled out as explanations for this observed association.
A recent study using data from the Avon Longitudinal Study of Parents and Children (ALSPAC) attempted to unpick this issue by investigating the association between levels of IL-6 at age 9 and depressive symptoms at age 18 (Khandaker et al., JAMA Psychiatry. 2014). This study found evidence of an association between increased IL-6 at age 9 and greater depressive symptoms at 18 (adjusted β=0.06, se=0.03)).
We expanded on this study using Mendelian randomisation (MR), an approach largely free from issues such as confounding and reverse causality. MR uses genetic instruments to proxy a modifiable risk factor and can thus strengthen causal inference using observational data.

Allelic risk scores for the IL-6 receptor have previously been developed. Following a recent genome-wide association study, we extended the score to increase instrument strength (F=87.4). The updated score contained 4 SNPs from the *IL6R*, *TDRD10* and *ABO* genes.
Although the estimate was in a consistent direction with the observational result, we found little evidence of a causal association. This will be expanded to a meta-analysis incorporating similar studies to improve precision of the causal estimate.
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PM09.038

Seizures And Attention Deficit As Unique Clinical Hallmarks In A Patient With Digeorge Syndrome.

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DiGeorge syndrome (DGS, OMIM 188400) is one of the most common chromosomal disorders, with an estimated prevalence of 1 in 4 000-6 000 live births. It is actually a well-defined primary immunodeficiency disorder classically associated with abnormal facial appearance, congenital heart defects, hypoparathyroidism with hypocalcaemia, as well as cognitive, behavioral and psychiatric problems. Pathological hallmarks include conotruncal abnormalities and absence or hypoplasia of the thymus and parathyroid glands. The facial appearance of patients with DGS is characterized by hypertelorism, micrognathia, short philtrum with fish-mouth appearance, antimongoloid slant, and telecanthus with short palpebral fissures. However, not all patients with DGS show these typical dysmorphic findings and the diagnosis can be delayed for many years. It has been reported that children and adults with DGS have high rates of behavioral, psychiatric and communication disorders. Here, we report the case of a patient that was 11 year-old boy born to unrelated healthy parents. Initially, he was admitted to the Neuropaediatric Department by seizures, attention deficit and socialization problems. The brain computed tomography CT revealed calcification located in the left basal ganglia. The EEG was abnormal due to generalized intermittent slow waves without any epileptiform discharges. Genetic analysis by MLPA technique (P245 MCR-Holland) showed a microdetion in 22q11 affecting to GP1BB, CLDN5 and SNAP29 probes, associated to a DiGeorge Syndrome. Clinical absence of the typical hallmarks of this syndrome should not exclude this disease due to variable phenotypes. Continuous efforts to define diverse clinical manifestations of this syndrome will lead to proper evaluation and intervention for patients.

PS09.039

DNA methylation of CACNA1C in bipolar disorder

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Introduction: Genome-wide levels of association between bipolar disorder (BPD) and schizophrenia (SZ) have been reported with the *CACNA1C* gene. This gene encodes the pore-forming unit of L-type voltage-gated calcium channels. The associated SNPs are located in a cluster in intron 3, however the functional effects of the associated SNPs remain to be determined. In this study we investigated if DNA methylation of *CACNA1C* in blood differed between BPD patients compared to healthy control subjects.
Material and methods: DNA methylation status of five CpG islands (CGIs) in the *CACNA1C* gene was investigated using Sequenom EpiTYPER in 630 bipolar disorder patients and 340 control subjects.
Results: Four out of five *CACNA1C* CGI islands were either fully methylated or fully unmethylated. In contrast one CGI showed intermediate methylation levels with substantial intra-individual variation. Follow-up analysis of this CGI identified a significant difference in DNA methylation levels between the BPD cases and healthy controls. Furthermore the methylation status of this CGI was highly correlated with the genotypes of the BPD and SZ risk SNPs.
Conclusion: The functional effects of the *CACNA1C* GWAS risk alleles may be mediated through changes in methylation status of intron 3.

PM09.040

Postnatal microcephaly in Dyggve-Melchior-Clausen syndrome is associated with hypomyelination and neuronal trafficking defects

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Dyggve-Melchior-Clausen syndrome (DMC, MIM #223800) is a rare inherited dwarfism characterized by spondyloepimetaphyseal dysplasia, postnatal microcephaly and intellectual disability, and caused by loss-of-function mutations in the DYM gene encoding DYMECLIN, a Golgi-associated protein involved in intracellular trafficking. Interestingly, both skeletal growth defects and microcephaly develop during childhood and are therefore rarely diagnosed at birth. This suggests that the physiological mechanisms affected in DMC involve postnatal processes. We used Dymeclin-mutant mice to determine the cause of microcephaly and to identify defective mechanisms at the cellular level. Brain weight and volume were reduced in all mutant mice from postnatal day 5 onward. Mutant mice displayed a narrowing of the frontal cortex, although cortical layers were normally organized. Interestingly, the corpus callosum was markedly thinner, a characteristic we also identified in three unrelated DMC patients carrying the p.Gln483Ter nonsense mutation. Consistent with this, the myelin sheath was less compact and not properly rolled, while the number of mature oligodendrocytes and their ability to produce myelin basic protein were significantly decreased. Finally, cortical neurons from mutant mice and primary fibroblasts from DMC patients displayed substantially delayed endoplasmic reticulum to Golgi trafficking, that could be fully rescued upon Dymeclin re-expression. These findings indicate that Dymeclin is crucial for proper myelination and anterograde neuronal trafficking, two processes that are highly active during postnatal brain maturation.
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PS09.041

Molecular genetic investigations of a Norwegian dystonia cohort by gene-panel based next generation sequencing

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Dystonia is a group of clinically heterogeneous neurological disorders characterized by involuntary movements and prolonged muscle contraction, which may involve the entire body or only an isolated area. Several genetic forms of dystonia have been identified, but genetic testing has been limited by time-consuming and costly procedures. However, using next generation sequencing technology, it is now possible to develop inexpensive tests by which multiple genes can be screened in parallel.
We designed a panel including the coding sequences of 17 genes implicated in different forms of dystonia, using a similar method as described previously (Pihlstrøm L, *et al*., Ann of Hum Genet, 2014). We screened these genes in 116 clinically well-characterized Norwegian dystonia patients as well as 120 ethnically matched controls. The sequencing yielded high quality data with average 98% bases covered above 80x in the targeted intervals. Our bioinformatics pipeline identified 11 variants for further validation and confirmation. We identified variants in *ANO3*, *CIZ1*, *GCH1*, *NKX2-1*, *PNKD*, *PRRT2*, *SGCE*, *TOR1A*, and *TH*. Out of these, two confirmed variants are truncating changes located in *GCH1* and *CIZ1*. After the ongoing validation analysis, we will be able to establish a molecular diagnosis in a subset of patients.
Our approach of gene-panel based next generation sequencing will be able to gauge the potential of the method used and will point towards future directions for targeted screenings in such heterogeneous neurological disorders. Moreover, such fast, cost-effective, and robust screening methods will help improve molecular diagnosis, genetic counseling, and therapeutics.

PM09.042

Next Generation Sequencing in neurogenetics - our experience

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Next Generation Sequencing (NGS) methods transform our approach in genetic testing of neurological disorders, particularly in the area of single gene diseases. NGS helps us to improve diagnostics of well known disorders, to detect rare mutations in genetic heterogeneous diseases, and to discover new disease causing genes. Growing number of experiences indicate huge NGS benefits, as well as some method limitations.
We describe here results of NGS method implementation in three different single gene/familiar diseases in patients from Serbia. We have analyzed: 1. one family with early onset dystonia - parkinsonism, negative for common DYT and PARK mutations, 2. one family with cerebral congenital cavernous malformation, negative for CCM1 gene mutations, 3. one sporadic case of Wilson disease with only one heterozygous ATP7B mutation (H1069Q) detected by screening of five mutation - prone exons. Mentioned DYT, PARK, CCM1 and ATP7B analyses were performed in Genetic laboratory of Neurology Clinic CCS, Belgrade, by Sanger's sequencing of targeted regions on ABI3500 Genetic Analyzer. NGS was performed on Illumina MiSeq platform using TruSight One panel. After bioinformatic analysis of NGS data we detected novel GIGYF2 (PARK11) gene variant (p.E531V) in dystonia - parkinsonism family and novel CCM2 gene variant (p.Y118X) in family with congenital cavernous malformation. Both changes are confirmed by Sanger sequencing and recognized as likely pathogenic by software's prediction. However, in the case of Wilson disease, NGS confirmed known mutation but did not detected second ATP7B gene change. Our results are good illustration of power and challenges of NGS methods.

PS09.043

Detection of disease-causing mutations in cases of unexplained early-onset ataxia by using panel based next-generation sequencing

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Spinocerebellar ataxias (SCAs) in patients with an onset age under 30 years (early-onset ataxias, EOAs) are mostly caused in autosomal recessive genes and show a wide range of clinical symptoms. Due to the large variability and overlap of phenotype and the rapidly increasing amount of (often very rare) ataxia related genes, it is challenging to find a molecular diagnosis in these patients.
The study cohort of 131 cases with EOA was negatively screened for dominant repeat expansion SCAs and FRDA. Patients with a genetic mutation sufficient to explain the ataxia or with neurodegenerative disease in more than one generation were excluded beforehand. Samples were processed with a custom-built ataxia specific HaloPlex panel (Agilent) for 132 genes and sequenced on a MiSeq (Illumina) followed by bioinformatics analyses using our in-house pipeline.
By this approach, on average, >95% of the target region was covered ≥20x with a mean coverage of 257 ± 71 reads. The analysis of filtered variants identified 18 patients (14%) with clear pathogenic biallelic mutations in 9 genes (e.g. 7x *SACS*, 1x *PLA2G6*, 1x *PNPLA6*, 2x *NPC1*, 2x *SYNE1*), 8 patients (6%) with probably pathogenic mutations in 6 genes (*SETX*, *SYNE1*, *ITPR1*, *KIF5C*, *SACS*, *TTPA*) and additional 8 patients (6%) with a potential de-novo mutation in a dominant ataxia gene (*ITPR1*, *PRKCG*, *SPTBN2*, *CACNA1A*).
Disease specific panel sequencing thus enables a highly effective, cost-efficient and fast detection of underlying mutation which could facilitate therapeutic treatment of symptoms or precise prediction of the course of the disease.

PM09.044

Early Infantile Epileptic Encephalopathies - a Center Audit

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Introduction: Epileptic encephalopathies (EE) are specific age-related brain conditions with a detrimental effect of continuing seizures and electrographic discharges on the function of the developing brain. Early Infantile Epileptic Encephalopathies (EIEE) are EE with onset before age 3-4 months. Etiologies are heterogeneous and whole-exome sequencing (WES) techniques can help to clarify their etiopathogenesis.
Patients&Methods: Patients were retrospectively selected based on their clinical and electroencephalographic diagnosis of EIEE, registered at the Epilepsy Clinic of our Tertiary Hospital from 1995 to 2013. Exclusion criteria were: brain injury secondary to hypoxic-ischemic encephalopathy, trauma, stroke, infection and intracranial hemorrhage. We performed a descriptive analysis concerning demographics, epileptic phenotype and investigation. Selected patients without diagnosis are under WES analysis using TruSeq capture followed by sequencing on a HiSeq2500.
Results: From the 27 selected patients, median age for first manifestation was 45 days [1day-4months]. Six patients had electroclinical syndromes (four Ohtahara syndrome; two early myoclonic encephalopathy). The majority had severe DD. Brain MRI of 22 patients was diagnostic in nine (eight had malformation of cortical development, MCD). Six (22%) patients died. Ten patients (37%) had a confirmed molecular diagnosis (six metabolic diseases, three genetic syndromes, one MCD). All were sporadic situations. NGS analysis' results of eight patients will be presented at the Conference.
Conclusion: Our work reflects the metabolic etiology's impact in this group of EE, their clinical and etiologic heterogeneity. Children with EIEE have elevated mortality (22%) and morbidity (100%) rates. An accurate etiologic diagnosis for such disorders is helpful for an effective individual management, genetic counselling and sometimes therapeutic intervention.

PS09.045

Massively Parallel Sequencing in the Epilepsies: New gene pathways and challenging clinical dogma

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Purpose:
We set out to find the genetic cause of focal epilepsy in a family with individuals with lesional and non-lesional focal epilepsy, as detectable by MRI analysis. The family showed autosomal dominant inheritance and genome wide linkage analysis failed to identify a linkage region.
Methods:
We carried out exome capture and massively parallel sequencing on two individuals from the family who were affected with focal epilepsy and analysed the data using an in-house bioinformatics pipeline. Candidate causative genetic variants were validated by direct Sanger sequencing and were analysed for co-segregation with affected status and assessed for likely pathogenicity.
Results:
We identified a mutation in *DEPDC5* as being causative of lesional and non-lesional focal epilepsy in the family. We then identified *DEPDC5* mutations in additional families with mutation-positive individuals with lesional and non-lesional focal epilepsy. *DEPDC5*-associated malformations include bottom-of-the-sulcus dysplasia and focal subcortical band heterotopia.
Conclusion:
We have found that mutations in *DEPDC5* cause familial cases of focal epilepsy associated with structural brain lesions as well as in cases with no structural brain abnormalities. We have therefore shown that lesional and non-lesional epilepsy can have a shared genetic aetiology. This challenges the long held view that lesional and non-lesional epilepsy are distinct clinical entities with different causes. DEPDC5 negatively regulates the mTOR pathway which plays a key role in cell growth and metabolism. The clinical and radiological phenotypes associated with *DEPDC5* mutations share features with the archetypal mTORopathy, tuberous sclerosis, raising the possibility of new therapeutic avenues for patients.

PM09.046

Mouse Neuronal epileptic phenotype analysis using MEA platform.

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Epilepsy is a neurological disorder that affects about 3% of the population and is characterized by sudden and violent seizures (focal or generalized). Different causes have been associated with epileptic disorders (brain injury,

stroke or brain damage at the time of birth) and in recent years, several genes mutations have been implicated in this complex disorder. However the mechanisms that connect many of the identified mutations/genes to epilepsy are still unknown.
We focused our attention on the *hnRNPU* gene in which two different loss-of-function *de novo* mutations (chr1:245019921 CATTTGTCTTT/C INDEL; chr1:243086781 C>T) have been identified in two different patients affected by epileptic encephalopathy. *hnRNPU* has an important role in mRNA processing, transcription and splicing. To better understand the neurobiological role of *hnRNPU* in epilepsy we used a Micro-electode array (MEA) platform that is able to record the field potential of electrically active cells from 768 electrodes simultaneously. We plated *wild-type* mouse cortical neurons on MEA plates and used the shRNA to knock-down *hnRNPU* gene in a half of the tested cells. Next, we constructed a tool to analyze the complex multi-dimensional output of the MEA recordings. This enabled us to observe a general effect of decreased expression on neuronal activity of treated neurons. Preliminary results specifically show that the *hnRNPU* knock-down results in characteristic changes in neuronal activity, where treated cells have prolonged bursts activity with increased firing rate compared to the wild, revealing a neuronal phenotype activity that can be compared with what is observed in-vivo in epileptic seizures.
Internal Funds

PS09.047

Microelectrode array analysis captures seizure-like activity due to down-regulation of microRNA-128

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Microelectrode arrays (MEAs) are powerful tools for detecting spontaneous neuronal activity. MEAs have been used to detect the response of primary dissociated neurons to neurotoxins and are emerging as reliable drug screening platforms. Recent studies indicating the involvement of microRNAs in epilepsy phenotypes encouraged us to investigate the effect of modulating microRNA-128, on neuronal activity. Mice deficient in microRNA-128 are prone to fatal seizures. Modulating microRNA-128 in the MEA system allowed us to directly test whether MEAs are useful to screen other microRNAs that influence risk of seizures. In particular, we sought to evaluate whether down-regulation of microRNA-128 results in clear excitability phenotypes by MEA analysis. We used primary cortical neurons from wild-type postnatal day zero C57BL6 mice and a lentivirus delivered microRNA sponge to target the mature microRNA-128. Sponges are competitive microRNA inhibitors which express tandem repeats that are partially complementary to the microRNA sequence. We recorded neuronal activity on the MEA for 15 minutes each day to collect spike, burst, and network synchronicity data. Our data show that down regulation of microRNA-128 by inhibition with a sponge results in significantly increased neuronal activity. These features are consistent with the previous observations that microRNA-128 deficiency promotes neuronal excitability. These experiments illustrate the utility of the MEA platform in evaluation of microRNAs in regulation of network phenotypes.

PM09.048

Molecular characterization of a cohort of 352 patients sheds light on genetic heterogeneity in early onset epileptic encephalopathy.

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We have studied a cohort of 352 patients suffering from early onset epileptic encephalopathy. This cohort was studied either in a diagnostic or in a research setting. All patients had epilepsy beginning before 3 months of age, abnormal interictal EEG, no brain abnormality and a normal metabolic screening. They all had abnormal neurological development. Globally, among the 352 patients, we identified 75 probably damaging mutations (21,3%). As a first step, all patients were screened for mutations in the KCNQ2 or STXBP1 genes. We identified 33 mutations in KCNQ2 (9,3% of the cohort) and 19 mutations in STXBP1 (5,4% of the cohort). If we consider the diagnostic cohort only (189 patients) the yield is 17,4% for KCNQ2 and 10% for STXBP1 mutations. All mutations in these two genes were de novo but two, which have been inherited from mosaic carriers.
In the research cohort (163 patients), we found mutations in the KCNT1, SCN8A, TBC1D24, SCN2A, KCNQ3, SLC13A5, GABRG2, CDKL5 and GRIN2A genes. High resolution arrayCGH analysis was also performed for 109 patients in the research cohort. This analysis revealed numerous rearrangements, most of which were inherited from the healthy parents, although they had not previously been reported in genomic variant databases. Several pathogenic rearrangements were also found including submicroscopic deletions of STBXP1, MEF2C, WDR45 and SCN2A. These results, together with our current strategy aiming at increasing the diagnostic yield, will be detailed at the conference.

PS09.049

Testing epileptic encephalopathy candidate genes in additional samples shows paucity of recessive hits

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Aim: In recent studies of mutations underlying epileptic encephalopathies, numerous genes have been identified with unsufficient statistical support. We sought to increase support for at least some of those genes.
Methods: samples from 362 patients with epileptic encephalopathy were tested on a panel of 370 known and candidate genes. Thirty-eight patients had similarly affected sibs, 37 sporadics had consanguineous parents, 287 sporadics had no known parental relatedness. The genes had been collected from literature and earlier experiments in our labs. Eighteen genes had OMIM-annotations for epileptic encephalopathy; 66 genes were expected to be recessive, 288 dominant, 13 X-linked, the remainder unknown. In the first screen, no parents were included. We looked for novel coding variants.
Results: Thirty-eight patients had probably pathogenic variants in OMIM-annotated genes. One inbred patient was homozygous for a recessive candidate gene, four sporadic patients were compound heterozygous for recessive candidate genes. Some genes, e.g. *NFASC*, had more novel hits than expected from its length, while some genes with a known connection to severe epilepsies were NOT overrepresented relative to gene size. Of the genes with excess novel hits *COL7A1* and *FREM2* are known causes for the recessive diseases Epidermolysis bullosa and Fraser syndrome respectively.
Conclusions: Despite a likely enrichment for recessive forms of epileptic encephalopathy, through inclusion of multiplex and inbred families, very few patients had homozygous or compound heterozygous mutations in recessive candidate genes, suggesting that recessive epileptic encephalopathies are very rare. Sequencing of our patients' parents and further analysis will sort out new EE-genes.

PM09.050

A genetic study of Primary Familial Brain Calcification and Paroxysmal Kinesigenic Dyskinesia

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Introduction: Primary Familial Brain Calcification (PFBC) also known as Fahr's disease is a rare genetic disorder including heterogeneous and progressive neurologic dysfunction, caused mainly by calcification in basal ganglia. Thus far, 3 genes have been identified to be involved in Fahr's disease, SLC20A2, PDGFRB and PDGFB. Recent evidence suggests that SLC20A2 might be associated with Paroxysmal Kinesigenic Dyskinesia (PKD).
Materials and methods: We performed a SLC20A2, PDGFRB and PDGFB gene analysis of 4 families with Fahr's disease and a SLC20A2 screening of a PKD family. The genomic DNA was extracted, genes amplified and then sequenced.
Results: Screening for SLC20A2 detected a novel missense mutation c.1618G>A in heterozygous form in two affected members of a PFBC family. This mutation leads to a Gly540Arg substitution in a highly conserved residue in transmembrane domain IX. A novel SLC20A2 deletion in heterozygous state c.21_21delG (p.Leu7Fs*10) was found in a patient of a second PFBC family.
Conclusion: As predicted the first mutation causes a loss of function of the protein while deletion causes a frameshift generating a truncated protein. SLC20A2 gene mutations shape the impairment of the uptake of Pi. In conclusion our study confirms that SLC20A2 mutations are mainly responsible for PFBC.
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2. Min Zhu, Xuan Zhu, Hui Wan, Daojun Hong. Familial IBGC caused by SL-

C20A2 mutation presenting as paroxysmal kinesigenic dyskinesia. Parkinsonism Relat Disord. 2014 Mar; 20 (3):353-4

PS09.051

A modulatory trans-effect on age-at-onset in familial amyloid polyneuropathy (FAP) ATTRV30M in Portugal

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Introduction: Early and late-onset forms of familial amyloid polyneuropathy (FAP) ATTR V30M (aka Val50Met) are not separate entities: they may coexist in the same family, with offspring often showing anticipation of age-of-onset (AO). Our aim was to identify modifiers closely linked to the TTR locus that may in part explain the wide variability in AO.
Methods: Eight variants at the TTR locus were analysed, in a sample of 722 individuals (588 V30M carriers). An intensive in silico analysis was performed to study a possible regulation of gene expression.
Results: Haplotype A was the most common in our population. Noteworthy, haplotype C was more frequent in early-onset carriers than in late patients (p=0.012). When we compared allelic frequencies of each SNP of haplotype C between very early (≤30yrs) and late-onset cases (≥50yrs), we found a significant association of the A allele of rs72922947 with early-onset (p=0.009), which remained significant after a permutation-based correction. Also, the GA genotype was associated with a decrease in mean AO of 8.6 yrs (p=0.014).
We also found interesting, unreported results in the in silico analysis. Several alterations in the mechanism of splicing, transcription factors binding and miRNAs binding were observed.
Conclusions: We found a common haplotype linked with V30M mutation and, importantly, a possible modulatory effect on AO by a genetic variant present in the normal chromosome. These putative mechanisms of regulation of gene expression, within the TTR gene, could be potential therapeutical targets.

PM09.052

Familial Fatal Insomnia in a large Portuguese family

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Fatal familial insomnia (FFI) is a rare human prion disorder, clinically characterized by progressive insomnia and dysautonomia preceding motor and cognitive deterioration. FFI is linked to the mutation D178N in the PRNP gene and to the presence of a methionine codon at position 129 of the mutant allele (D178N-129M haplotype).
We report the case of a 49-year-old man presenting with insomnia, attention deficits and sexual dysfunction. The following month he started having unsteady gait, behavioral changes and was unable to work due to executive dysfunction. Over the subsequent months he became agitated, with stereotyped behavior and vocalizations, and rapidly deteriorated, dying 10 months after onset.
In the investigation, EEG showed bursts of frontal slow waves, but no periodic activity was detected, and polysomnography revealed a normal pattern with the presence of sleep spindles and K complexes; MRI revealed mild fronto-temporal atrophy; CSF analysis was negative for 14-3-3 protein; necropsy study revealed a mild fronto-temporal atrophy, severe neuronal loss and astrogliosis in the thalamic nuclei, but no spongiform degeneration; Western blot analysis for protease-resistant prion protein did not detect the presence of the abnormal protein in frontal cortex. Finally, PRNP gene sequencing revealed a heterozygous D178N mutation associated with a homozygous methionine polymorphism at codon 129.
This patient's family has an autosomal dominant pedigree with several carriers and affected members, although with a phenotypic variability related, at least partly, to the specific polymorphism at codon 129 of the non-mutant allele of PRNP gene.

PM09.054

Friedreich ataxia and Limb-girdle muscular dystrophy type 2A segregating in a single pedigree from Bulgaria

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Friedreich ataxia (FRDA, MIM 229300) is an autosomal recessive form of ataxia, caused by mutations in the *FXN* gene (MIM 606829), localized at 9q21.11. Typically, the disorder is associated with an instable *GAA* trinucleotide expansion in intron 1. Homozygous expansion accounts for 98% of the cases of FRDA. However, about 2% of the FRDA patients are compound heterozygous for the expansion and a point mutation in the *FXN* gene.
Limb-Girdle Muscular Dystrophy Type 2A (LGMD2A, MIM 253600; Calpainopathy) is another autosomal recessive neuromuscular disorder presented with progressive proximal (limb-girdle) muscle weakness. LGMD2A is caused by mutations in the *CAPN3* gene (MIM 114240) localized at 15q15.1.
Here we report a single pedigree affected by these two different recessive neuromuscular disorders, namely FRDA and LGMD2A. The molecular genetic testing showed that in this family FRDA is caused by an expanded GAA repeat in compound heterozygous state with a novel point mutation c.442C>T, p.Gln148* in the *FXN* gene. This represents the first report of Bulgarian FRDA case carrying a point mutation in the *FXN* gene. The LGMD2A affected individual was found to be compound heterozygous for the two most common *CAPN3* mutations for the Bulgarian population (c.550delA, p.Thr184Argfs; c.967G>T, p.Glu323*).
To the best of our knowledge we are reporting the first Bulgarian family with two neurological disorders caused by mutations in genes on different chromosomes and segregating independently in the family. This single pedigree with four different mutations is an example for the high genetic heterogeneity of the Bulgarian population.

PS09.055

Fast detection of genetic variations in frontotemporal dementia using next-generation sequencing-based on a custom AmpliSeq™ panel and Ion Torrent PGM sequencing.

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Frontotemporal dementia (FTD) is a syndrome characterized by profound behavioral changes and degeneration of the frontal and anterior temporal cortex and it is a clinically, pathologically and genetically heterogeneous disorder. Many candidate genes are associated with FTD. To set up a fast and comprehensive assay in order to determine genetic diagnosis in FTD, we employed the standard next-generation sequencing (NGS) workflow. We designed a custom AmpliSeq™ panel for sequencing the most prevalent FTD-causing genes (PSEN2, TARDBP, PSEN1, FUS, GRN, MAPT, APOE, PRNP, and APP) using the Ion Torrent Personal Genome Machine (PGM) Sequencer. Our AmpliSeq custom panel allowed us to efficiently explore 99% of targeted sequences. Few patients with a clinical diagnosis of FTD was screened to identify the disease-causing mutations. Using adjusted alignment settings, all genetic variants present in covered region were readily identified. We found in a patient two heterozygous mutations in two genes (Cys139Arg in GRN gene, and Glu318Gly in PSEN1 gene) previously described pathogenic. In all patients we identified the common genetic variant in the 3' untranslated region (3'UTR) of GRN gene (rs5848; c.*78C>T), associated with sporadic FTD, and several synonymous variants, classified not pathogenic, in the other genes. In conclusion, these results suggested that our NGS approach based on an ampliseq custom panel is a highly efficient, and can readily identify FTD-causing mutations that are associated with high phenotypic variability.

PM09.056

Mutations in PGRN, MAPT and C9ORF72 in Polish patients with frontotemporal lobar dementia

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Introduction
Frontotemporal lobar degeneration (FTD) is the second most common form

of presenile dementia. FTD, is clinically, pathologically and genetically heterogeneous disorder. Up to 40% of FTD patients report family members with FTD supporting the important contribution of genetic factors. Seven genes are linked to FTD, with the most prevalent mutations located in *PGRN*, *MAPT* and *C9ORF72*.

Materials and Methods

The study group comprised 138 FTD patients (mean age of onset 56,3±11,8; 57 patients with familial FTD) and control group consisted of 257 neurologically healthy persons. *MAPT* and *PGRN* were direct-sequenced. Hexanucleotide repeat expansion located in *C9ORF72* was analyzed using repeat–primed PCR.

Results

In the group of FTD patients fifteen putative pathogenic mutations (six in *MAPT*, four in *PGRN*, and five in *C9ORF72*) were identified including three novel mutation: *MAPT* G55R, *PGRN* D317fsX11, and *PGRN* P439_R440fsX6. Mutation in three analyzed genes are responsible for 11% of all analyzed FTD cases and 31% of familial cases.

Conclusions

Although the mutations in the analyzed genes are responsible for a significant percentage of FTD cases from the Polish population, it is recommended to apply new generation sequencing methods (NGS) to detect mutations in the remainder FTD cases.

PS09.057

Progranulin levels in repeated blood withdrawals and cerebrospinal fluid in (pre)symptomatic frontotemporal dementia caused by progranulin mutations

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Introduction: Frontotemporal dementia (FTD) is the second most common form of presenile dementia characterized by progressive behavioral and/or language disorders. Mutations in progranulin (*GRN*, autosomal dominant inheritance), are causative for this disease in 5 -10 percent of cases, leading to reduced levels of progranulin protein levels due to haploinsufficiency. Upcoming therapeutic trials focus on enhancing progranulin production. To be able to correctly interpret effects of these agents, knowledge of natural variability and correlations between progranulin levels in blood and cerebrospinal fluid are essential.

Methods: We studied the variability of progranulin levels in 42 subjects (4 FTD patients with *GRN* mutation, 19 presymptomatic *GRN* carriers and 19 healthy controls). We performed lumbar punctures in 28 subjects (3 FTD patients with *GRN* mutation, 16 presymptomatic *GRN* carriers and 9 healthy controls).

Results: Progranulin levels are significantly lower in *GRN* carriers compared to healthy controls (mean 7866 pg/mL versus 27473 pg/mL, p=0.00). Progranulin levels in plasma are remarkably stable over time (mean variability per subject is 11%). Presymptomatic versus symptomatic carriers show equal median progranulin levels and variability (p=0.29 resp. p=0.26). In CSF, progranulin levels are also significantly lower in carriers (median 334 pg/mL) compared to controls (median 761 pg/mL, p=0.00). Plasma progranulin levels show a good correlation with CSF progranulin levels in the total cohort (Spearman's rho=0.82, p=0.00).

Conclusions: Peripheral progranulin levels are stable and correlate well with central levels, therefore plasma progranulin levels are a promising biomarker for subjects with *GRN* mutations.

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PS09.059

Expanding the Clinical Spectrum of GABRG2 Mutations

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Gamma-aminobutyric acid (GABA) receptors are a family of proteins involved in the GABAergic neurotransmission of the brain. GABRG2 is a member of the GABA-A receptor gene family of heteromeric pentameric ligand-gated ion channels. Heterozygous mutations in the *GABRG2* gene have been found in the patients with childhood absence epilepsy, familial febrile seizures, and generalized epilepsy with FS plus. We analyzed whole exome sequencing in patients with neurodevelopmental disorders. We report two patients with atypical manifestations with novel *GABRG2* mutations.

Patient 1

This 5 year-old girl was born to nonconsanguineous healthy parents. Her developmental milestone was delayed from early infancy. She cannot walk alone. She showed severe intellectual disability (ID) and autism spectrum disorder (ASD). Microcephalus and dysmorphic features were noted. *De novo* heterozygous mutation in the *GABRG2* gene (p.A106T) was identified. EEG showed sporadic spikes. Epileptic seizure was not observed.

Patient 2

This 5 year-old boy was born to nonconsanguineous healthy parents His developmental milestone was delayed from early infancy. He cannot sit or walk. He showed severe ID and generalized epileptic seizures. He constantly moved his extremities. Microcephalus and dysmorphic features were noted. *De novo* heterozygous mutation in the *GABRG2* gene (p.R270G) was identified.

We suppose that mutations in the *GABRG2* gene may be responsible for ID and ASD. Patient 1 lacked seizures. Clinical spectrum of *GABRG2* mutation was expanded.

PM09.060

The emerging utility of genetic testing in clinical psychiatry

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There is ample evidence that psychiatric disorders have a strong genetic basis. Family history is the strongest predictor of increased disease risk. Increasing knowledge about the genetic architecture in a substantial subset of patients with severe mental illness urges revising the clinical standard of care. We inform the field about the state of the art of clinical genetics applied to psychiatric practice. Furthermore, we present our findings in a cohort of patients with severe mental illness and comorbid mild to moderate intellectual disability, where in a considerably number of the cases a molecular diagnosis was provided, and give clinical guidelines as to when it is useful to request a clinical genetic evaluation in the setting of psychiatric care. Clinical genetic testing and counselling is warranted in patients with complex psychiatric syndromes, and especially in those with mental retardation and/or multiple congenital abnormalities and/or a positive family history.

PS09.061

Clinical and molecular-genetic characteristics of two Gerstmann-Straussler-Scheinker patients.

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Gerstmann-Straussler-Scheinker disease is the rare autosomal-dominant disease with a prevalence of 1 in 10 million. We herein present a description of clinical and molecular-genetic characteristics of two unrelated probands with Gerstmann-Straussler-Scheinker syndrome in three generations.

Patient 1, a 35 years old man, complained at constrained muscles, change of speech, memory impairment. By 34 years dysarthria and memory impairment became evident. EEG showed moderate violation of bioelectric activity of a brain in the form of dysfunctions the diencephalic structures. MRI of a brain didn't show significant changes. Similar symptoms were observed in the proband's mother and brother, who died at the age of 37 and 38 years, respectively, 3 years after of the first symptoms. DNA - analysis identified the previously described mutation Ala117Val in the PRNP gene.

In Patient 2, a 43 years old women, the first symptoms of deasease noticed in 40 years when the severe ataxia revealed. In the neurologic status symptoms of static and dynamic cerebellar ataxy in combination with pyramidal symptoms were dominat. There was no expressed cognitive frustration, the patient complained only at reducing of memory on the current events. EEG and MRI of a brain didn't show significant changes. Similar symptoms were observed in the patient's mother and grandmother who died during 5 and 7 years, after the first symptoms were discovered. DNA- analysis identified the previously described mutation Pro102Leu in the PRNP gene.

These results confirm the opinion of many authors about so-called „telencephalic" type of disease at patients with a mutation Ala117Val in contrast to the „atactic" type observed at Pro102Leu mutation.

PM09.062

Genetic characteristics of hemiplegic migraine in Finnish migraine families

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Introduction: Hemiplegic migraine (HM) is a rare subtype of migraine with aura. Typical HM attacks are characterized by fully reversible motor weakness that is associated with visual, sensory or speech symptoms. Furthermore, attacks may occur with basilar-type symptoms, prolonged hemiplegia, confusion, coma, fever or seizures. To date several mutations have been identified for HM locating in three ion-transporter genes: *CACNA1A, ATP1A2* and *SCN1A*.

Materials and Methods: In order to study the involvement of the three HM genes in the Finnish HM patients we have exome sequenced (Agilent/Illumina: Sanger Institute) 303 patients from 252 families fulfilling the criteria for HM. The mean sequence depth of the target exome was 86. The minor allele frequencies of exonic variants were checked against The Exome Aggregation Consortium (ExAC) and 1000G project data. Validation and segregation analyses of all novel and rare exonic and splice site variants are ongoing.

Results: Exome sequencing analyses revealed 43 potentially functional, unknown, low or rare frequency variants in the three FHM genes. One of the identified variants was the well-known mutation - p.Thr666Met - in *CACNA1A*. The variant was found in one family segregating perfectly.

Conclusions: Most of the so far studied variants do not seem to segregate with the HM phenotype. Hence, after validation and segregation analyses we will study whether different variant composition including e.g. several less deleterious variants, could predispose to HM susceptibility. In addition, the exome data will be screened genome-wide to identify whether variants in other genes could contribute to HM.

PS09.063

Uniparental disomy causing hereditary spastic paraplegia: Paternal disomy in FA2H causing homozygous SPG35 in two non-consanguine families

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Hereditary spastic paraplegias (HSPs) are degenerative diseases of upper motor neurons characterized by a progressive spastic gait disorder. They are genetically highly heterogenous. SPG35 is an autosomal-recessive (AR) HSP caused by mutations in the fatty acid 2-hydroxylase (FA2H), which is essential for maintenance of the neuronal myelin sheath. Thus far approx. 20 mutations have been described in 13 SPG35 families with 36 affected patients.

Genetic screening was performed by whole exome sequencing in patient #1 and by a HaloPlex assay providing high coverage of all known HSP genes ("HSP panel") in patient #2. MLPA was used to screen for FA2H deletions and microsatellite marker analysis by PCR to identify uniparental disomy (UPD).

We detected different novel homozygous FA2H mutations in two non-related non-consanguine families. Segregation analysis revealed both fathers being heterozygous mutation carriers whereas both mothers did not carry FA2H mutations. We presumed a maternal macro deletion within SPG35 to be causing a hemizygous genotype, but a MLPA assay failed to confirm our hypothesis. Finally, a microsatellite array revealed paternal disomy in both families leading to homozygous SPG35 mutations.

UPD has rare been described as causative mechanism in neurodegenerative diseases. This is the first report of UPD as a cause for HSP. Interestingly, we identified this rare mode of inheritance in two families with the rare genotype SPG35. We are not aware of any mechanism how SPG35 mutations may trigger UPD.

Since UPD seems to be a relevant factor in AR HSP we recommend segregation analysis especially in non-consanguine homozygous index cases to unravel further UPD cases in this field.

PM09.064

Identification of the first Sudanese families carrying novel mutations in SPG11, SPG57 and SACS genes

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Spinocerebellar neurodegeneration results in a spectrum of clinically and genetically heterogeneous syndromes that frequently impose a diagnostic dilemma. Pure forms of hereditary spastic paraplegias (HSP) and hereditary ataxias (HA) represent both ends of the spectrum. Screening of 74 known spasticity culprit genes was performed on 51 patients from 21 extended Sudanese families with multiple consanguinity loops. Genetic diagnosis was established in 5 families with autosomal recessive complex spastic ataxia. SPG11 nonsense [exon 34: c.6349G>T / p.Glu2117*, exon 21: c.3568A>T / p.Lys1190*] and frameshift [exon 36: c.6709del / p.Ala2237Gln*7] mutations were identified in 3 families with HSP with atrophy of the corpus callosum. Two other families carried homozygous mutations in SACS gene [exon10: c.7739G>A / p.Trp2580*] and in SPG57/TFG gene [exon 2: c.64C>T / p.R22W].

The c.6709del SPG11 mutation was previously reported in one Somalian patient with spastic ataxia but with slightly different clinical presentation including the age at onset (2 years versus 10-17 years in Sudanese patients). All other 4 mutations were novel.

The family with TFG missense mutation (p.R22W/ PB1 highly conserved domain) is the second SPG57 family worldwide. It presented with motor rather than the visual impairment presentation of the first SPG57 Indian family (p.R106C / coiled-coil domain). The Sudanese SPG57 family had multiple MRI abnormalities (and uniquely hemorrhages in one patient) as seen in collagen disorders.

This study shows the great genetic heterogeneity of HSP in Sudan and demonstrates that more genes will come in the future to explain the nosology of these diseases.

PS09.065

TFG mutation hot spot causes varied hereditary spastic paraplegia phenotypes

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The hereditary spastic paraplegias (HSPs) are a clinically and genetically heterogeneous group of degenerative motor neuron disorders in which the cardinal clinical feature is progressive lower limb spasticity. This feature may occur in isolation ('pure' HSP), or be accompanied by other neurological or non-neurological clinical features ('complicated' HSP). We investigated the genetic basis of disease in two unrelated families, one with pure HSP, and the second with a complicated HSP associated with optic atrophy and neuropathy. Genome-wide SNP mapping in both families identified a single notable region of homozygosity peculiar to affected family members on chromosome 3q12.2, indicating that the disease gene was likely located in this region in each family. Exome sequencing in an affected individual from the pure HSP family identified a homozygous missense mutation (p.Arg106His) within the *TFG* gene, located within the mapped region. Subsequent analysis of the *TFG* gene in the complicated HSP family revealed another sequence alteration, again affecting arginine 106, although this time resulting in its substitution with cysteine (p.Arg106Cys). Thus both sequence alterations result in the substitution of the highly conserved arginine 106 residue with two different amino acids with distinct chemical properties, indicating that the clinical severity of disease may relate to the differing chemical properties of the substituted amino acid. Taken together, our findings confirm a causative role of *TFG* mutation in HSP and identify residue arginine 106 of *TFG* as a mutation hotspot to be considered for diagnostic screening in both pure HSP as well as complicated forms of HSP. This research is supported by MRC grant G1002279.

PM09.066

Spastic paraplegia: massive sequencing of 74 genes reveals a world of missing genes to uncover

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Hereditary Spastic Paraplegias (HSPs) are a heterogeneous group of neurodegenerative disorders characterized by lower limb spasticity and weakness. Genetically, HSPs are complex with four modes of inheritance described and with >60 genes identified. Approximately 60% of the families persist without molecular diagnosis reinforcing their genetic heterogeneity.
In Portugal, a cohort of 98 families with spastic paraplegia remained without molecular diagnostic. In order to identify the disease-causing mutation in these families, 2 custom sequencing kits were developed to search for mutations in known genes, covering 34 and 74 genes, respectively, that included some genes causing a similar phenotype and some candidate and newly identified genes found by the SPATAX partners.
In these 98 families we identified the responsible gene in 22, resulting in a frequency of 22,5%. This low frequency could be explained by the fact that these families were the ones remaining after the exclusion of the most frequent HSP genes. Variants with unexpected inheritance modes and phenotypes were found in known genes. Our results show the importance of testing families with all modes of inheritance for the same set of genes and also, the importance of testing genes causing overlapping phenotypes.
These results suggest that there is still a large set of genes responsible by spastic paraplegia and probably more mechanisms leading to the disease to be uncovered.

PS09.067

Genetic testing for Huntington's Disease in consanguineous families

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The Yorkshire Regional Genetics Service has contact with all patients who undergo predictive and diagnostic genetic testing for Huntington's Disease (HD) within the NHS in North, East and West Yorkshire, as well as some areas of Northern Lincolnshire. HD occurs in all ethnic groups, but is more prevalent in the European population.
Our local population includes a significant proportion of individuals of South Asian origin. Genetic counselling and genetic testing in families from this group present different challenges to those encountered in other populations. In a recent study of our Bradford population, 28% of babies were born to consanguineous parents (18% were born to parents who were first cousins). In consanguineous families there is a risk that individuals with HD may be homozygous for the disease-causing CAG expansion. Homozygous expansions do not significantly alter the clinical phenotype in HD, but if detected on genetic testing this would significantly affect the offspring risk (which would be 100% for developing HD rather than the 50% expected following autosomal dominant inheritance). A homozygous result would also confirm that both parents of the proband are at risk of HD, when perhaps only one parent is aware of their diagnosis.
We present examples of consanguineous families where at least one individual has been diagnosed with HD. We will discuss the genetic counselling issues raised, and the approach taken to the reporting of genetic testing in individuals at risk of being homozygous for an HD expansion.

PM09.068

Making a new diagnosis of Huntington's Disease; the experience of a UK regional genetics service.

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The Yorkshire Regional Genetics Service has contact with all patients who undergo genetic testing for Huntington's Disease (HD) on an NHS basis in North, East and West Yorkshire, and parts of North Lincolnshire. This cohort includes patients, referred by neurologists or psychiatrists, in whom a diagnosis of HD is suspected on clinical grounds but who have no known family history of the condition.
We have reviewed the data of individuals tested over the last 10 years and noted an increase in the number of people diagnosed with HD in the absence of a known or assumed family history of the condition.
In our experience, these families present different counselling challenges to those encountered in families already known to be affected by HD. For example, adult children of a person diagnosed with HD in later life may perceive HD as a benign condition affecting only older people, even when genetic anticipation has been explained. When a new diagnosis is made in an older adult, not only has the tested individual completed their family, but often their own offspring are in the midst of child-bearing or have completed their families too. This can mean that decisions about predictive testing, reproductive choice, and disclosing information about the diagnosis may be faced over a shorter time period than in people who have grown up with the knowledge of their HD risk.
Here we present our data and discuss the challenges created by making a new diagnosis of HD in a family.

PS09.069

Association analysis of narcolepsy without cataplexy and idiopathic hypersomnia

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Narcolepsy without cataplexy (NA w/o CA) is a lifelong disorder characterized by excessive daytime sleepiness and REM sleep abnormalities, but does not exhibit cataplexy. Several studies have reported that NA w/o CA is associated with human leucocyte antigen *HLA-DQB1*06:02* similar to narcolepsy with cataplexy (NA-CA). The sample sizes of the studies were small because NA w/o CA is an infrequent condition. We have therefore formed a collaborative research group to promote the study. In this study, we examined *HLA-DQB1* in 146 Japanese patients with NA w/o CA and 1,418 controls. The frequency of *DQB1*06:02* in the patients was significantly higher than that in the controls (allele frequency: 16.8% vs. 7.6%, $P = 1.6\times10^{-7}$, OR = 2.40). After controlling for the effect of *DQB1*06:02*, distributions of *HLA-DQB1* alleles were compared between NA w/o CA and NA-CA to assess whether the genetic backgrounds of the two diseases have similarities. The distribution of *HLA-DQB1* alleles in *DQB1*06:02*-negative NA w/o CA was significantly different from that in NA-CA ($P = 8.4\times10^{-7}$). On the other hand, the patterns of the *HLA-DQB1* alleles were similar between *DQB1*06:02*-positive NA w/o CA and NA-CA. *HLA-DQB1* analysis was also performed in 171 Japanese patients with idiopathic hypersomnia (IHS). No significant associations were observed. The findings suggest that *DQB1*06:02*-positive NA w/o CA has an autoimmune pathogenesis in common with NA-CA, but *DQB1*06:02*-negative NA w/o CA and IHS might be unique from NA-CA. We also performed a genome-wide association study to identify genetic markers associated with *DQB1*06:02*-negative NA w/o CA and IHS. We will discuss the results of the study.

PM09.070

Exome sequencing identifies a novel SNX14 mutation and confirms a new syndrome with intellectual disability and cerebellar atrophy

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Clinical exome sequencing has open possibilities for diagnosis of previously unrecognized genetic disorders. Here we describe a consanguineous family with two children presenting with global developmental delay and subtle dysmorphic features, accompanied by cerebellar atrophy and small vermix, originating from Saudi Arabia.
By whole exome sequencing and family analysis we identified a novel mutation in the SNX14 gene (c.2722C>T, p.Gln908*), which introduces a premature termination codon in exon 27 of the gene. This mutation is located in the PX associated domain C (PXC) of the SXN14 protein and will likely cause a loss of function or lead to nonsense mediated decay. This novel mutation was found in homozygosis in both affected children and the parents were confirmed to be heterozygote carriers. Two unaffected siblings were also heterozygote carriers of this mutation confirming co-segregation of the disease and the p.Gln908* mutation.
Recently, Thomas et al. described a new syndrome in three consanguineous families from Portuguese and Turkish descent in whom homozygous mutations were identified in the SXN14 gene. The mutations (partially) remove the

RGS, PX or PXC domains and were shown to lead to loss of function which is also the pathogenic mechanism suspected in our family. Our results confirm that homozygous mutations in the SNX14 gene cause a new syndrome with intellectual disability and cerebellar atrophy. Retrospective exome analysis of cases with this specific phenotype is recommended.

PS09.071

New genetic makers in young ischemic strokes

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Strokes are today one of the leading causes of mortality, as well as of subsequent serious long-term physical and mental morbidity, among patients in many different countries all over the world. Ischemic stroke (IS) is a complex genetic disorder caused by a combination of multiple genetic and environmental factors and incidence rates for IS increase exponentially with increasing age but 10-15% of patients are under 45 years of age. When atherosclerosis is the leading cause of strokes in the elderly, there are multiple etiologies in young (arterial,cardiac,genetic...) and nearly half of strokes is of unknown cause. The aim of our work is to search risk and genetic susceptibility factors contributing to the occurrence of IS in a french young population (300IS<40 years) compared with 300 young controls. First results show that hypertension, migraine, tobacco and drugs are significant risk factors for ischemic strokes in our young population (OR=35, 3.8, 4 and 2.8, respectively). Then, we tried to identify genomic susceptibility loci using array-CGH approach. Some copy number polymorphisms (CNP) observed, a deletion may be candidate for a protective role in IS. We also observe uncommon CNV, some of them being particularly reccurent in patients or already associated with strokes like ALOX5AP ou PRKCE. We also applied a candidate-gene approach on coagulation genes and a significant association was found for the C677T in the MTHFR gene (5,10-methyltetrahydrofolate) and young IS (OR=2.39, p=0.02 for TT genotype). In conclusion, this study confirmed the implication of environmental and genetic factors in ischemic strokes before 40 years and suggests new genetic risk factors for IS.

PM09.072

A comprehensive molecular diagnostic service for Joubert syndrome and related disorders

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Joubert syndrome (JS) is an autosomal recessive ciliopathy characterised by the molar tooth sign on MRI, hypotonia and developmental delay with or without oculomotor apraxia and breathing abnormalities. Joubert syndrome and related disorders (JSRD) describes individuals with JS and additional clinical features including retinal dystrophy, renal disease, hepatic fibrosis and polydactyly. JSRDs are genetically heterogeneous with at least 27 causative genes described that encode proteins of the primary cilium or centrosome. Molecular diagnosis enables accurate prognosis and disease management based on known genotype-phenotype correlations; e.g. hepatic fibrosis (*TMEM67*) or nephronophthisis (*NPHP1, RPGRIP1L* and *CEP290*).
Our diagnostic service for JSRD has evolved from a targeted Sanger Sequencing and MLPA screen in 6 genes to a NGS approach using Agilent's Haloplex Targeted Enrichment system to capture all known JSRD genes. This capture system is very flexible; the initial NGS panel of 18 genes was increased to 21, and currently comprises 27 genes. Next generation sequencing is undertaken on Illumina's MiSeq platform.
Of the 100 patients that have undergone testing a molecular diagnosis has been made in 42%. Pathogenic mutations have been identified in the following genes: *AHI1*, *ARL13B*, *C5ORF42*, *CC2D2A*, *CEP290*, *INPP5E*, *NPHP1*, *OFD1*, *RPGRIP1L*, *TCTN1*, *TCTN2*, *TMEM231* and *TMEM67*.
A service update and a selection of interesting cases will be presented. These include a *TCTN2* single exon deletion identified using NGS coverage data, a Joubert patient with two *NPHP1* frameshift mutations and 2 cases that employed RNA analysis to support pathogenicity of *TMEM67* splice variants.

PS09.073

Identification of a novel locus for large-vessel ischemic stroke: a genome-wide association study

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Background: Ischemic stroke is a major cause of death and disability in the world. A major ischemic stroke subtype (large artery atherosclerosis [LAA]) has been suggested to have some genetic components in individuals of European ancestry. However, whether genetic predisposition to LAA varies by ethnicity remains unknown.
Methods and Materials: We sought to identify novel genetic variations that predispose individuals to large-vessel ischemic stroke by using a genome-wide association study (GWAS) in 444 individuals with LAA and 1,727 controls in a Han Chinese population residing in Taiwan. We replicated the study in an independent Han Chinese population comprising an additional 319 cases and 1,802 controls. We identified five SNPs, including rs2415317 (P = 3.10 × 10−8), rs934075 (P = 4.00 × 10−9), rs944289 (P = 3.57 × 10−8), rs2787417 (P = 1.76 × 10−8), and rs1952706 (P = 2.92 × 10−8), at one novel locus on chromosome 14q13.3 within PTCSC3 (encoding papillary thyroid carcinoma susceptibility candidate 3) that were associated with large-vessel ischemic stroke at genome-wide significance (P < 5 × 10−8).
Conclusions: The newly identified SNPs within PTCSC3 gene for LAA are not only found for the first time to reach genome-wide statistical significance but also locate in a risk locus correlated with papillary thyroid carcinoma (PTC), which strongly suggest the need of future studies on the association between LAA and PTC. Our findings provide insights into the genetic basis of large-vessel ischemic stroke and identify a novel pathway that might be applicable for future therapeutic intervention. Grant support: Academia Sinica Genomic Medicine Multicenter Study, Taiwan (40-05-GMM).

PM09.074

The hunt for genes in undetermined Leukodytrophies

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Leukodystrophies are a heterogeneous group of orphan genetic diseases that affect the white matter (WM) and its main component, the myelin. Despite the large number of mutations and disease-related genes identified, 60% of families remain without genetic marker. We performed Exome sequencing in 75 patients selected from consanguineous family, non-consanguineous family with more than one affected children and few sporadic cases with specific clinical presentation We identified disease-causing mutations in 56% of cases. Our cohort was subdivided in three groups 15% genes already known in leukodystrophies, 9% genes known in leukoencephalopathy with atypical clinical presentation and 32% potential candidate variants, functional analysis are currently performed. For the remaining 44% of cases the variants identified need further analysis. This study underlines the role of Exome sequencing in the diagnosis of leukodystrphies by resolving diagnostic confusion arising from atypical or incomplete presentations and identification of new disorders.

PS09.075

A novel mutation in *LGI1* gene found in a series of consecutive epileptic patients with auditory aura

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Introduction: Autosomal Dominant Lateral Temporal Epilepsy (ADLTE) is characterized by focal seizures with a typical involvement of auditory aura. Auditory symptoms occur during seizures that are produced by the primary or association auditory cortices within the lateral temporal lobe. The disease may onset both in juvenile and adult terms, where good management is usually accomplished through antiepileptic drug treatments. Mutations in

leucine-rich glioma-inactivated 1 (*LGI1)* gene have been implicated in ADTLE both in familial and sporadic forms.
Material and Methods: All coding exons of *LGI1* were screened for mutations in a cohort of 26 individuals affected with ADTLE. Physical and neurological examinations were performed and detailed information on family history was collected for all affected individuals.
Results: A heterozygous novel variant resulting in a missense change within the LRR (Leucine rich repeats) domain of LGI1 protein was detected in one individual with a negative family history for epilepsy. This variant was not present in his parents and two unaffected sisters suggesting a *de novo* change. The pathogenicity of our novel variant is supported by its evolutionary conservation, its absence in 289 control individuals and *in silico* protein prediction tools.
Conclusions: Identification of rare *LGI1* gene mutations in sporadic cases is especially important for genetic counseling, supporting diagnosis, therapeutic decisions and understanding the pathogenesis of the disease. Screening of *LGI1* in independent patient series is required in order to establish genotype-phenotype correlations that will assist relevant diagnostic testing.
This study supported by IU-BAP and TUBİTAK (Project numbers 26263 and 113S331.)

PM09.076

Is the *MACROD2* gene a potential risk factor for developing ASD?

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Autism spectrum disorders (ASDs) are common, affecting 1% of children in the US population with a 4:1 male to female ratio, and have a strong genetic basis; yet the cause of about 70-80% ASDs remains unknown. *MACROD2* gene, on chromosome 20p12.1, is one of the several genes previously associated with risk for autism (Anney et al., Hum Mol Genet 2010, 19: 4072-4092). We report on two brothers aged 8ys3m and 9ys6m, respectively meeting the DSM-5 clinical criteria for Autism Spectrum Disorder. Both were assessed on the ADI-R and the ADOS-2. Each child underwent a cognitive assessment by the Wechsler Intelligence Scales, depending on the age. Both had a low average total IQ, and did not show congenital malformations or minor anomalies. An array-CGH showed, in both, an interstitial deletion on the short arm of chromosome 20 (20p12.1), sizing 72 Kb, with paternal segregation. The deletion encompassed the *MACROD2* gene. This was associated in the younger brother with an interstitial duplication on 16q21, sizing 366 Kb, with paternal segregation, encompassing the *RPS15AP34* gene; and, in the older one, with an interstitial deletion on 7p21.2, sizing 135 Kb, with maternal segregation, encompassing no genes. The father reported significant impairments in verbal and non-verbal communication and social interactions with his peers during childhood and adolescence; associated with specific difficulties in mathematics.
Our observation seems to strengthen the role of the *MACROD2* gene as a potential risk factor for developing ASD.

PS09.077

Megalencephalic leukoencephalopathy with subcortical cysts (van der Knaap disease): study of two families

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Introduction: Megalencephalic leukoencephalopathy (MLC) with subcortical cysts is an infantile-onset inherited autosomal recessive disease. Patients typically show macrocephaly, motor abnormalities, seizures, and almost constant late-onset mild mental deterioration. Brain magnetic resonance imaging (MRI) reveals specific patterns in the anterior temporal and frontoparietal regions. The MLC1 gene is responsible of this disease. Mutations in this gene have been identified in families from different ethnic backgrounds.
Objective: In this context, we report two Tunisian families having children affected with MLC. Indirect genetic analyzes showed a transmission profile for the mutant MLC1 gene. Patients and methods: It is about two Tunisian consanguineous families. The first family shows a girl and a boy with macrocephaly, seizures, ataxia and MRI confirmation. The second family has four daughters; of which, one had clinical and radiological signs for MLC. Genotyping by analysis of four microsatellite markers surrounding or inside the MLC1 gene, helped to establish the haplotype of each individual and to follow the transmission of haplotype associated with the disease.
Results: the indirect study by genotyping revealed the presence of a homozygous haplotype associated with the disease among all affected children in the two families. Discussion and conclusion: The diagnosis of MLC is based on a combination of clinical and radiological features and requires a genetic confirmation. The indirect study by genotyping is important to show the type of haplotype transmission among patients. This is expected to be followed by sequencing of MLC1gene looking for variation. This MLC genetic study could be important for prenatal diagnosis and mental deterioration prevention by therapy development.

PM09.078

MicroRNA expression profiling of methamphetamine dependence in rat hippocampal tissue

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Metamphetamine is a highly addictive psychostimulant that induces behavioral changes, the hippocampus being the part of the brain that plays the important role in these changes, especially in drug addiction. However, little is known about the underlying mechanisms of methamphetamine effects on global miRNA expression. The objective of this study was to determine the global miRNA profiling of the methamphetamine dependence from the rat hippocampal tissue and to identify the miRNAs which are associated with methamphetamine use and dependence. The study comprised of 18 male rats which were divided into 3 groups: continuous methamphetamine treatment (0.5,1,2,3,4,5,5.5mg/kg), single dose acute methamphetamine treatment (5.5mg/kg), and a control group. Addiction behavior was determined using Conditioned Place Preference task. The analysis of the miRNA profiling was performed using Affymetric microarray GeneChip® System. For behavior test, we found that the addiction behavior only occur with continuous treatment of methamphetamine, but not in acute treatment. Differential profiling of miRNAs indicated that 30 miRNAs were significantly up-regulated and 1 down-regulated when the acute treatment was given; 40 miRNAs were up-regulated and 1 down-regulated in the continuous methamphetamine treatment group. Comparing between acute treatment without addiction and the continuous treatment with addiction, 29 miRNAs were up-regulated with 1 down-regulated for addiction phenotype. The miRNAs were selected when there are more than 2 times fold changes, ANOVA test with $p<0.05$ and FDR test with $p>0.05$. Conclusion, our results suggest that dynamic changes occur in the expression of miRNAs, which may be associated with the methamphetamine use and dependence.
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PS09.079

Molecular and clinical delineation of the 2p15-16.1 microdeletion syndrome and proposal of a new candidate gene for microcephaly

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Interstitial 2p15-p16.1 microdeletion is a rare microdeletion syndrome previously reported in 14 patients. It is characterized by moderate to severe intellectual disability, autism spectrum disorder, microcephaly, short stature, dysmorphic features, structural brain anomalies and multiple congenital organs defects. It is considered a contiguous gene syndrome involving deletion of several genes. Deletions previously reported are variable in size (from 203 kb to 6.9 Mb) and have non recurrent breakpoints. We report here three additional patients carried a 2p15-p16.1 microdeletion shown by SNP-array analysis (Cyto12-SNP, Illumina) : a prenatal case and two postnatal cases. The prenatal and one of the postnatal cases share an overlapping 2p15p16.1 deletion of 105 kb including only XPO1 and the distal end of USP34. Both patients presented features overlapping the clinical spectrum of the 2p15p16 microdeletion syndrome including dysmorphic facial features and brain structural abnormalities. We confirm that one or both genes are probably involved in facial dysmorphic features, cognitive impairment and brain structural abnormalities observed in 2p15p16.1-deletion syndrome.
The third patient we reported on is a 4 years-old male with an heterozygous de novo a 427 kb deletion at 2p16.1 (chr2:60624940-61051867) containing BCL11A and PAPOLG and a phenotype characterized by speech delay, autistic traits and stereotyped behavior but without microcephaly.
Considering previous deletions in the 2p15p16.1 region and our three new cases, we precise the genotype-phenotype correlation of the microdeletion syndrome. Moreover we suggest that the REL gene could be considered as a candidate gene for microcephaly.

PM09.080

A new dominant form of microcephalic primordial dwarfism

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Primary microcephaly (PM) is a rare and heterogeneous group of affections characterized by a reduction in brain volume and intellectual disability. In some circumstances, PM is also associated with primordial dwarfisms (microcephalic primordial dwarfisms, such as Seckel, MOPD2 and Meyer Gorlin syndromes). Interestingly, all microcephalic primordial dwarfisms reported to date are recessively inherited and genes identified so far encode centrosomal proteins. However, they account for less than half of cases in Europe, indicating that other genes remain to be identified.
Here, we report on a new form of microcephalic primordial dwarfism with an autosomal dominant inheritance, in which no known PM gene was found to be mutated.
The father (25 years old) and his daughter (3 years old) shared common features in their medical history: Intrauterine growth retardation followed by postnatal growth failure (length -4 SD), microcephaly (-5 SD), and dysmorphic features including microretrognathia, hypotelorism, shortening of fore-arms and legs and muscular hypertrophy. In addition, the father had a micropenis, a hypoplasia of the scrotum and developed an early onset cataract at the age of 7. The daughter had a cleft palate, and feeding difficulties that needed nutritional support until the age of 2. Her cranial MRI showed isolated microcephaly.
This case report enlarges the frontiers of microcephalic primordial dwarfism to dominant forms that are likely linked to impairments of new specific PM genes.
This study was supported by PHRC (P100128) and ANR (ERARE2-2013).
Verloes A et al. Am J Med Genet 1997;68: 455-460
Verloes A et al. GeneReviews® 2013

PS09.081

A functional miR-124 binding-site polymorphism in IQGAP1 affects human cognitive performance

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As a product of the unique evolution of the human brain, human cognitive performance is largely a collection of heritable traits. Rather surprisingly, to date there have been no reported cases to highlight genes that underwent adaptive evolution in humans and which carry polymorphisms that have a marked effect on cognitive performance. IQ motif containing GTPase activating protein 1 (IQGAP1), a scaffold protein, affects learning and memory in a dose-dependent manner. Its expression is regulated by miR-124 through the binding sites in the 3'UTR, where a SNP (rs1042538) exists in the core-binding motif. Here we showed that this SNP can influence the miR-target interaction both *in vitro* and *in vivo*. Individuals carrying the derived T alleles have higher IQGAP1 expression in the brain as compared to the ancestral A allele carriers. We observed a significant and male-specific association between rs1042538 and tactile performances in two independent cohorts. Males with the derived allele displayed higher tactual performances as compared to those with the ancestral allele. Furthermore, we found a highly diverged allele-frequency distribution of rs1042538 among world human populations, likely caused by natural selection and/or recent population expansion. These results suggest that current human populations still carry sequence variations that affect cognitive performances and that these genetic variants may likely have been subject to comparatively recent natural selection.

PM09.082

Variants in the NR3C2 gene as possible genetic predisposition for the development of multiple sclerosis

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Multiple Sclerosis (MS) is an autoimmune disease of the central nervous system caused by a complex interaction between multiple genes and environmental factors. We report on the incidental observation of two variants in the NR3C2 gene, involved in type 1 pseudohypoaldosteronism (PHA), in young MS patients and investigated whether NR3C2 might be a susceptibility gene for MS and autoimmune disease.
Case 1 is a male 12 years-old MS patient harboring a 112-181 kb deletion in NR3C2. He had no symptoms of PHA but serial blood investigations confirmed a mild elevation of aldosterone. Segregation analysis revealed that his mother and maternal aunt, both of which developed autoimmune thyroiditis, carried the same microdeletion.
Case 2 is the mother of a child with PHA. She presented at age 28 with MS and was known to carry the same NR3C2 splice-site mutation (c.1757+1G>C) as her daughter. The mother had elevated aldosterone levels.
In an independent cohort of 100 MS probands, NR3C2 sequencing detected a loss-of-function mutation (p.Ser585X) in a single patient with early-onset MS and a positive familial history. She had no symptoms of PHA but a mild reproducible elevation of aldosterone.
Our data suggest that NR3C2 loss-of-function variants may associate with a predisposition for developing autoimmune disease, particularly for MS. Though further validation of our findings in a larger cohort is necessary, the involvement of the renin-angiotensin-aldosterone system in the pathogenesis of MS may have important implications in counseling and management of patients with NR3C2 mutations and opens novel perspectives for MS treatment.

PS09.083

Increased first exon usage of THEMIS in resting CD4+ T-cells is associated with a genotype that is protective against the development of multiple sclerosis

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Genome wide association studies and subsequent fine mapping have identified a number of loci containing genetic variants associated with susceptibility to multiple sclerosis (MS), including the THEMIS/PTPRK locus on chromosome 6q. At this locus, associated SNPs are predominantly intergenic, suggesting that they may have a role in the regulation of gene expression. Both THEMIS and PTPRK are indispensable for the thymic production of CD4+ T cells, a process known to be defective in MS. The aim of this study was to investigate the functional mechanisms underlying SNP rs13204742, identified in the fine mapping Immunochip study as being the most significantly associated SNP at the THEMIS/PTPRK locus.
PBMCs were isolated from 73 genotyped, healthy individuals, obtaining near-equivalent numbers from each genotype at SNP rs13204742. The biological significance of this variant was investigated by studying genotypic effects on both total and isoform-specific expression of THEMIS, PTPRK and RP11-103C16.2 (an antisense gene to PTPRK), in ex vivo and stimulated CD4+ and CD8+ T-cells by quantitative PCR. Genotypic effects on thymic activity were determined by measuring sjTRECs and circulating CD4+ recent thymic emigrants (CD4+CD45RA+CCR7+CD31+ T-cells).
In ex vivo CD4+ T-cells, there was a significant decrease in THEMIS first exon usage with an increasing genetic load of the risk allele at SNP rs13204742 ($p=0.0055$, ANOVA). This difference was absent upon T-cell stimulation. Alternative first exon usage is a mode of regulating gene expression in a cell specific manner. Therefore our findings suggest that SNP rs13204742 may influence the risk of developing MS by affecting CD4+ T-cell specific THEMIS expression.

PM09.084

Results of targeted NGS strategy for the diagnosis of NBIA in a cohort of 71 patients

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In the expanding field of neurodegeneration with brain iron accumulation (NBIA), the development of sensitive brain imaging played a major role to characterize iron-related damages in patients. In the past years, new genes have been identified allowing genotype-phenotype correlations. However, overlapping phenotypes and non-informative pedigrees are still sources of delayed diagnosis.
For these reasons, a Next Generation Sequencing analysis was developed and tested in our series of 71 patients, comprising 32 patients in addition to the 39 patients previously analysed for FTL and PANK2 mutations by Sanger sequencing, including 3 FTL and 2 PANK2 diagnosed patients. Seven additional NBIA genes (ATP13A2, CP, C19orf12, DCAF17; FA2H, PLA2G6

and WDR45) were included in a custom panel of 35 genes dedicated to our routine molecular diagnosis platform. Two libraries of amplicons were designed with the AmpliSeqTM technology. Sequencing was performed on a PGM. The Torrent Suite (Life Technologies), SeqNext (JSI) and AlamutTM interface (Interactive Biosoftware) were used for read alignment, variant calling and interpretation respectively. Workflow analysis was validated on FTL and PANK2 positive patients. Recurrent artefacts were not considered. In silico, AmpliSeq design covered 99% of NBIA genes targeted regions, of which 98% were sequenced at least 40X. Uncovered regions were sequenced using Sanger technology.
Among the 32 additional patients, 1 CP and 1 PANK2 patients were diagnosed.
NGS is a powerful and cost-effective tool to search for mutations in heterogeneous genetic disorders but analysis effectiveness of known genes still remains disappointing in NBIA (10% molecular diagnosis, n=7/71).

PM09.086

Neurodegeneration with iron accumulation in brain (NBIA): Clinical assessment and genetic characterization by a means of a Spanish multi-center research network

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Neurodegeneration with brain iron accumulation (NBIA) is due to several genetic defects causing movement disorders and brain iron deposition in children, PKAN (pantothenate kinase associated neurodegeneration) being the most common one. Despite advances in the research of novel therapies for PKAN, validated international disease rating scales (DRS) to be used in clinical trials are lacking. We aim to identify and genetically characterize the Spanish population with NBIA and to design and validate a quantitative method for clinical assessment of PKAN patients within a cross-sectional multi-center project.
To date 43 NBIA patients from 20 Spanish hospitals have been recruited and assessed according to a new DRS for PKAN (PKAN-DRS) and genetic testing of the most frequent genes (PANK2, PLA2G6 and C19orf12) involved in NBIAs have been performed. Twenty-three patients have been diagnosed as PKAN and 11 patients carry mutations in the PLA2G6 gene. Moreover, 13 PKAN patients (median age 33 years) have been evaluated according to the PKAN-DRS (range 29-71). It is striking that the six Gypsy patients included in this research harbor the PANK2 p.T528 mutation in homozygosis which suggest a founder effect in our population, and present with lower PKAN-DRS scores for all subscales when compared with the group of patients with other mutations.
This cross-sectional multi-center has helped us to improve the diagnosis of NBIA patients. The proposal of a PKAN-DRS seems to be a reliable method mainly targeted to the evaluation of the neurological symptoms associated to PKAN.
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PS09.087

Neurodegeneration with Brain Iron Accumulation (NBIA): insights from gene coexpression networks

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Neurodegeneration with brain iron accumulation (NBIA) constitutes a group of neurodegenerative diseases characterized by a prominent extrapyramidal movement disorder, intellectual deterioration and a characteristic iron deposition in the basal ganglia. Ten genes have been identified so far, but limited information exists regarding expression and function of these genes within the human brain. To address possible relationships between known NBIA genes, predict their functions, and identify overlapping pathways, we used a systems-biology approach based on whole transcriptome gene expression analysis. As part of the UK Human Brain Expression Consortium (UKBEC), we analysed the expression profiles of 101 neuropathologically normal individuals (10 distinct brain regions each). Weighted gene co-expression network analysis (WGCNA) was used to cluster genes into co-expression modules. The overrepresentation of NBIA transcripts in basal ganglia modules (substantia nigra and putamen hereby studied) was assessed. Six NBIA-containing modules were found for the substantia nigra, but none was significantly overrepresented. Two putamen modules were significantly enriched for NBIA transcripts, namely the brown (PANK2, ATP13A2, C19orf12, COASY; P= 0.003) and green (FTL, DCAF17, FA2H; P= 0.021) modules. Enrichment analysis of these two putamen modules revealed an overrepresentation of gene ontology terms and KEGG pathways, including: brown module - synaptic vesicle endocytosis and axon cargo transport (biological processes), synaptic membrane (cellular component), and synaptic vesicle cycle KEGG pathway; green module - ensheathment of neurons, neuronal action potential and oligodendrocyte development (biological processes), myelin sheath (cellular component), and cadherin binding (molecular function). Our data suggests shared processes and pathways in NBIA gene networks.

PM09.088

Neurodegeneration and Brain Iron Accumulation: Clinical and genetic heterogeneity in the Hungarian population

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Neurodegeneration with Brain Iron Accumulation (NBIA) comprises a group of disorders characterized by progressive motor symptoms and cognitive decline. The genetic background of NBIA is heterogeneous. Not only the number of genes associated to the disease is increasing continuously, but the variety of the presenting clinical is increasing too.
Aim: The identification of the phenotype and genotype correlation of Hungarian NBIA patients in order to understand the dysfunction of proteins which are associated with NBIA and to collect genetic epidemiology data of the Hungarian NBIA patients.
Patients and Methods: We examined 20 patients having different extrapyramidal signs and cerebral brain iron accumulation confirmed by MRI (9 male, 11 female, mean age 35.1 years). The entire coding regions of the genes *PANK2, PLA2G6, MPAN* and *CP* were sequenced with Sanger method in the patients and in their family members.
Results: Pathogenic *MPAN* gene mutations were verified in 6, *PLA2G6* gene mutations in 3, *PANK2* gene mutations in 5, *CP* gene mutation in 3 patients. The NBIA syndromes were characterized by remarkable clinical heterogeneity. The clinical symptoms varied from the mild Parkinsonism over the isolated torticollis to severe dystonia.
Conclusion: In the Hungarian population the mutations of *MPAN* gene are more frequent compared to other populations. The symptoms caused by particular mutations of the single genes are not specific, although the extrapyramidal motor dysfunction with various degrees was observed in almost all cases. Both biallelic and heterozygous dominant mutations of the MPAN gene may result in clinical symptoms.
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PS09.089

Diagnostic yield of next generation sequencing in neurodegenerative diseases

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Neurodegenerative diseases are hereditary or sporadic disorders which are characterized by progressive nervous system dysfunction (e.g. dementias, Parkinson, Amyotrophic Lateral Sclerosis). Molecular diagnostics by Sanger sequencing is limited to testing common genes (e.g. *PARK2* for Parkinson) (there are too many rare genes to offer by Sanger testing). Therefore we developed a Next-Generation Sequencing (NGS) based workflow for DNA diagnostics for several neurodegenerative diseases (Ataxia: 49 genes; Dementia: 44 genes; Neurodegeneration with brain iron accumulation: 10 genes; Parkinson: 43 genes; Paroxysmal dyskinesia: 5 genes). This workflow was based on enrichment arrays (Agilent Sure Select) for all 151 genes and Miseq sequencing (paired-end, 150bp, Illumina). BAM-files are generated with an in-house analysis pipeline and variant calling is performed using SeqNext (JSI). The average vertical coverage for exons is ~300, very few fragments fail to reach the minimal required vertical coverage of 30. We were able to

detect deletions up to 65bp, insertions up to 29bp and somatic mosaicism. Sanger-sequencing was used to confirm the identified variants. Additionally, MLPA and repeat expansions tests were performed. We consider 12% of the cases solved (e.g. pathogenic PLA2G6 mutation and PLA2G6 exon 5-8 duplication). In 52% of the individuals an "unclassified variant" was found. Despite the use of in silico prediction programs, classification is difficult without additional clinical information, further patient/family studies and functional tests. In conclusion, the NGS-panels for neurodegenerative diseases are a powerful approach for DNA diagnostics. In order to increase the diagnostic yield, close collaboration between laboratory, clinical geneticist and referring specialist is required.

PM09.090

Estimating risk for neurofibromatosis type 1 among individuals with isolated café au lait spots

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Background: Neurofibromatosis type 1 (NF1) is caused by heterozygous mutations in the *NF1* gene. Isolated café-au-lait spots (CALS) are usually the first sign of NF1. However, not all individuals with isolated CALS will eventually be diagnosed with NF1. We aimed to estimate NF1 risk among individuals with isolated CALS.
Methods:Individuals with isolated CALS undergoing follow-up till age >6 years in a specialized NF center were included. NF1 status was determined based on mutation detection in blood and/or meeting the NIH disease criteria.
Results: Of 74 individuals with isolated CALS followed up in specialized NF center, 17 (23%) were eventually diagnosed with NF1. None had <6 CALS or atypical CALS at initial visit, as opposed to 67% and 33% respectively, amongst individuals without established diagnosis (P<0.001). Older paternal age (>40 years) was associated with NF1, while younger age (<30 years) was associated with non-NF1 (P<0.05). Genetic analysis performed in blood of children with isolated CALS at the MGL revealed disease causing mutations in 168/421 (40%). Among them, only 4.1% had <6 CALS compared with 28% among these without mutations (P<0.001). Mean age at testing was much younger among these with detected mutations: 1.47 years compared to 6.3 years for mutation negative patients (P<0.001). In addition, reported atypical CALS by referring physician was 6 times more common among these with detected mutations.
Conclusions: Estimation of risk for NF1 may be refined for individuals with isolated CALS based on clinical parameters
thus, may help with earlier management and follow up.

PS09.091

Whole genome sequencing and brain imaging of extreme phenotypes of Neuroticism in Korean women

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Neuroticism is a personality trait that has been associated with several psychiatric disorders, and considered one of the risk factors for developing major depression and anxiety disorders. It is a multifactorial trait influenced by the interaction between environmental and genetic factors. The aim of this study is to identify genetic variants contributing to Neuroticism using next-generation sequencing (NGS) technology, and associations between the genetic variants and variations of brain structure. We used an extreme phenotype study design to discover genetic variants and variations of brain structure for Neuroticism. We performed a whole genome sequencing and structural MRI of brain in 10 participants with two extreme of Neuroticism. Highly neurotic individuals showed decreased-volumes in the right dorsal premotor cortex and the left inferior frontal cortex comparing to lowly neurotic individuals. Whole genome analysis is in progress. We are going to link genomics with neuroscience to find individual differences in Neuroticism traits.
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PM09.092

Genetic Disorders in Arab Societies of Israel and the Palestinian Authority

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Rare inherited diseases often show complicated phenotypes and thus impose a relevant medical challenge. In Middle Eastern societies consanguinity is a deeply rooted cultural trait. This predisposes these societies for the occurrence of rare diseases with recessive traits. This study is an ongoing collaboration between physicians and scientists from Israel, the Palestinian Authority, and Germany aiming to investigate the molecular background of rare inherited diseases.
Patients were selected for inherited forms of neurological or ophthalmological disorders in 79 consanguineous families. Homozygosity mapping was performed using Agilent 250 K SNP arrays when applicable. Whole exon sequencing (WES) was done mainly using Agilents Sure Select All Exon V5 enrichment on an Illumina HiSeq2500 sequencer. Data analysis was accomplished using an in house bioinformatics pipeline.
Exome sequencing results in a large number of variants (>25.000), filtering for rare variants (Inhouse NGS database; 1000g; ESP6500) and for functional relevance reduced this count to <1.200. Due to the consanguineous background of the families, this amount could be further reduced comparing against mapping data or focusing on homozygous variants. An evaluation of the WES performance showed high coverage (~80% cov 20X) and target enrichment (~80X depth on target) parameters. Several disease causing mutations could be identified resolving the molecular background in 23 (~29%) families. For 16 families (~20%) promising candidate genes are under investigation.
WES offers an affordable and fast possibility to screen patients for disease causing variants. A consanguineous family background allows the discovery of mutations for neurodegenerative diseases in a high proportion of the patients.
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PS09.093

The contribution of Niemann-Pick SMPD1 mutations to Parkinson disease in Ashkenazi Jews

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Objective: To evaluate the prevalence of Ashkenazi three founder mutations in the sphingomyelin phosphodiesterase 1 (SMPD1) gene in Ashkenazi patients diagnosed with Parkinson's disease (PD); and their impact on PD phenotypic expression. Mutations in SMPD1 cause Niemann-Pick disease.
Methods: A case (n=287) control (n=400) study was undertaken following Institutional Review Board approval at Rambam Health Care Campus. All patients underwent physical, neurobehavioral, and neurologic examinations that incorporated the Unified Parkinson's Disease Rating Scale. Genetic testing for the three Ashkenazi mutations in SMPD1 gene was undertaken. The contribution of SMPD1 mutations to Parkinsons' clinical characteristics was studied on its own and elaborated subject to the presence of Ashkenazi founder mutations in the glucocerbrosidase (GBA) and the leucine-rich repeat kinase 2 (LRRK2) genes, both known to be associated with PD.
Results: Nine (3.1%) PD patients compared to two (0.5%) individuals from the control group were found to carry one of the three Ashkenazi SMPD1 founder mutations (c.996delC (fsP330), p.L302P and p.R496L) (p=0.007). The overall clinical characteristics of PD patients carrying SMPD1 mutations were similar to those of PD patients with no mutations in either of SMPD1, GBA and LRRK2 (n=189).
Conclusion: We maintain that disruptive mutations in SMPD1 constitute a risk factor for PD.

PM09.094

Molecular Analysis of Saudi Patients with Parkinson's Disease

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During the past two decades, a significant research effort was focused on dissecting the genetic architecture of complex neurodegenerative disorders including Parkinson's disease (PD). Linkage analysis and next-generation sequencing, facilitated identification of disease-causing mutations in more than six genes, while genome-wide association studies revealed several genetic susceptibility factors. Research into the genetics of PD was mainly conducted on American, European and Asian populations, whereas such research in Arab populations (excluding North African Arabs), specifically in Saudis, is lacking. This study aims to explore the genetic causes of PD in Saudis. A total of 85 PD-cases (sporadic and familial) were recruited and screened for potential pathogenic mutations in PD-established genes; *SNCA*, *PARKIN*, *PINK1*, *DJ-1*, *LRRK2* and other PD-associated genes; *UCHL1*, *GIGYF2*, *FBXO7*, *VPS35* using direct sequencing. Surprisingly, among the 105 variants detected, only a single potentially pathogenic mutation was identified in *PINK1* in two affected siblings. This is intriguing, as recessive monogenic-causes of the disease are anticipated to account for a significant portion of familial cases, given the consanguineous nature of the Saudi population and the high percentage (40%) of familial cases reported here. However, absence of potential pathogenic mutations in the ORF of the screened genes by no means exclude the possibility of gene expression/dosage alteration nor does it eliminate the possibility of the involvement of novel genes. We therefore, performed whole-exome sequencing on a subset of patients' samples. The analysis revealed a number of novel high priority candidate genes harboring potential pathogenic mutations, currently undergoing further validation.

PS09.095

Genetic mutations in early-onset Parkinson's disease in Hungarian patients

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Background: Mutations and copy number changes in *PARK2*, *PINK1*, and *DJ*-1 have been associated with autosomal recessive, *LRRK2* with autosomal dominant early-onset Parkinson's disease.
Aims: The estimation of the frequency of *PARK2*, *PINK1* and *LRRK2* mutations in Hungarian patients with early-onset Parkinson's disease.
Patients and methods: 140 Hungarian patients with early-onset Parkinson's disease were analyzed (men:women rate is 1:1, average age is 54). The complete sequences of *PARK2* and *PINK1* genes and the hot spots (28% of the coding regions) of the *LRRK2* gene were directly sequenced by Sanger method. Exon dosage was determined in the *PARK2* and *PINK1* genes by multiplex ligation-dependent probe amplification. The most common *LRRK2* mutation (S1647T) has been excluded by PCR-RFLP method.
Results: The presence of Q34R and R275W mutations in heterozygous genotype were found in 2 unrelated patients in the *PARK2* gene. The I803T mutation was found in heterozygous genotype in 1 patient in *LRRK2* gene. The common *LRRK* G2019S mutation was not present in the Hungarian patients, but a modifying factor (S1647T) was present in homozygous genotype in 11 patients. In the *PINK1* gene no pathogenic mutation was detected.
Conclusion: The prevalence of heterozygous mutations in *PARK2* observed in our cases support the concept that single heterozygous mutations in recessive Parkinson's disease genes may play a pathogenic role in the Hungarian patients as well. The analysis of the prevalence of the mutation frequencies of different genes associated to early onset Parkinson disease have important implications for genetic counseling of Hungarian patients with Parkinson's disease.
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PM09.096

Algorithms of MPS data evaluation in patients with Parkinsonism from genetically isolated population

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Introduction:
Parkinson's disease (PD) can be caused by genetic changes in many genes. The effect of these changes is determined by the nature of the mutation and ranges from weak associations to pathogenic mutation which leads to loss of protein function. Our study is based on epidemiological data which show significantly increased prevalence of PD (2.9%) in an isolated population of South-Eastern Moravia in the Czech Republic.
Objective:
Using MPS technology to compare our findings in DNA from 28 PD patients in the genes responsible for Parkinsonism (ADH1C, ATP13A3, EIF4G1, FBXO7, GBA + GBAP1, GIGYF2, HTRA2, LRRK2, MAPT, PARK2, PARK7, PINK1, PLA2G6, SNCA, UCHL1 and VPS35) to:
1) Already been described pathogenic mutations
2) Twelve control DNA samples from isolated population
Method of data assessment:
MPS data processing and trimming from FASTAQ through BAM to VCF files was done parallel by Ion Reporter and NexGene software.
Variants were than filtered using following parameters: AQ>20; Read coverage >20; MAF<0,002; SIFT: 0 - 0,1; PolyPhen: 0,15 -1; Grantham 6-215
Results:
After filtering out, there were found three missense mutations in LRRK2 gene: rs33995883 in 6/1 patient/control (p/c), rs33958906 in 1/1p/c, undescribed p.Ser633Phe in 3/0p/c, one missense mutation in MAPT gene rs63750072 in 6/1p/c and one mutation in HTRA2 rs72470545 in 3/1p/c.
Conclusion:
Our findings together with detailed clinical characteristics of patients could contribute to further understanding of PD molecular pathogenesis and to creating a clinically applicable diagnostic procedure.
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PS09.097

The PCSK6 intronic region associated with handedness controls expression of a novel shorter isoform

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We recently reported the first gene, PCSK6, associated with handedness at genome-wide significance level (P< 0.5*10-8, Brandler et al. 2013). A Variable Number Tandem Repeat (VNTR) within the same PCSK6 locus has been found to be associated with degree of handedness in an independent study (Arning et al. 2013). Interestingly, PCSK6 is known to activate NODAL, a morphogen involved in a highly conserved pathway known to regulate left/right body axis determination. Our previous data suggest these pathways controlling development of LR asymmetry in the body are reused for brain midline development, which in turn influence functional asymmetries such as handedness (Brandler and Paracchini 2014). All previous most significant associations fall within a linkage disequilibrium (LD) block containing a secondary promoter our data show to be bidirectional in nature and controlling transcription of both a novel PCSK6 isoform and a long non-coding antisense RNA. Taking forward previous GWAS findings, we have conducted a detailed functional analysis using a combination of further genetic analysis, in-silico predictions, and molecular assays. Our functional studies support an allele-specific effect on transcription factor binding affinity for a previous top-associated SNP rs11855425 however our genetic data could not find any evidence supporting a VNTR role. With this study we have dissected the molecular mechanisms underlying the PCSK6 association with handedness, indicating the regulatory effect the region has on specific RNA isoforms. Future work will focus on understanding the function of the novel shorter PCSK6 isoform and confirming the identity and role of the transcription factors binding at the secondary promoter.Neurodys018696, WT090532/Z/09/Z, MRCG090074791070, G1000569/1 and MR/J003719/1.

PM09.098

De novo mutations in sporadic Finnish patients with PEHO-like features

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PEHO syndrome (Progressive encephalopathy with Edema, Hypsarrhythmia and Optic atrophy; MIM 260565) is an autosomal recessive inherited progressive infantile encephalopathy. The main features of PEHO syndrome are hypotonia, infantile spasms and hypsarrhythmia, psychomotor retardation, absence or early loss of visual fixation, edema of the face and limbs, and typical dysmorphic features. A founder mutation in a novel gene underlies PEHO in 30 Finnish patients. A number of patients present with many, but not all, clinical features of PEHO, but are negative for mutations in the novel PEHO gene. This group is clinically heterogeneous suggesting genetic heterogeneity. To characterize the genetic background of these PEHO-like patients, we performed exome sequencing for 27 sporadic patients. In 4 cases also the parents were exome sequenced. As the group clinically overlaps with early-infantile epileptic encephalopathies, where new mutations account for a substantial proportion of patients, we focused on analysis of de novo mutations. Utilizing the Finnish exome database SISu, which included data from 3200 individuals, we were able to significantly restrict the number of candidate variants. We have identified a likely pathogenic mutation in 16 individuals, of whom 13 were exome sequenced without parents and three with parents. Of the 16 identified mutations 14 were validated to be de novo by Sanger sequencing in full trios. Four of the validated de novo mutations were in genes previously not associated with disease. Our data imply that exome sequencing combined with an ethnically matched reference database is an efficient method for identification of de novo mutations and does not necessitate sequencing of full trios.

PS09.099

PRX-related neuropathy with cataract: expansion of the clinical spectrum of perxiaxin associated neuropathies and identification of a novel mutation.

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Hereditary neuropathies are a heterogeneous group of congenital neurological disorders. The gene encoding the periaxin protein (PRX) is responsible for autosomal recessive early onset demyelinating neuropathy: Charcot-Marie Tooth disease 4F (CMT4F) or its severe form Dejerine Sottas neuropathy Periaxin protein is essential for maintenance of the peripheral nerve myelin. It also functions as a scaffold protein that plays a role in lens membrane organization . We present a boy diagnosed with hereditary neuropathy and cataract carrying a novel homozygous mutation mutations in the PRX gene. The proband is a 5 year old boy with normal cognition he had delayed motor milestones and subcapsular cataract. His healthy parents are of Muslim-Arab origin and unrelated. He has experienced frequent falls since early age, however, his motor deficiencies tend to ameliorate with time. Neurological examination revealed: weakness of the distal lower limbs muscles and absence of deep tendon reflexes. NCV demonstrated dramatic symmetrical reduction of sensory and motor velocity of the lower limbs, compatible with a demyelinating process. Whole exome sequencing of the boy's DNA revealed a novel homozygote frameshift mutation, c.418-419insG (p.M149 fs) in the PRX gene, confirmed by Sanger sequencing. The parents are heterozygote carriers of the mutation. Hence this seems to be the disease causing mutation in this boy. This is a new clinical manifestation of PRX-related hereditary neuropathies. Lens abnormality caused by mutations in the PRX gene were shown only in knock out mice. It is suggested that careful eye examination should be done in patients with congenital neuropathy in order to rule out PRX associated hereditary neuropathy.

PM09.100

Partial deletion of *SLC20A2* as a rare cause of PFBC

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Bilateral accumulation of calcium in the brain, most commonly in the basal ganglia, but also in the cerebellum, thalamus, and brainstem can be inherited in an autosomal dominant fashion and is then referred to as primary familial brain calcifications (PFBC). PFBC is a clinically and genetically heterogeneous disorder. Despite prominent calcifications some individuals remain asymptomatic while others become severely affected by motor, cognitive and psychiatric symptoms. To date, mutations in three genes (*SLC20A2*, *PDGFRB*, and *PDGFB*) have been linked to PFBC. Interestingly, a whole gene deletion of *SLC20A2* has recently been identified indicating that copy number variations may play a role in the pathogenesis of PFBC.

We screened eleven PFBC patients (four sporadic cases and seven patients from four families) for exonic deletions or multiplications in the *SLC20A2* gene. All patients were tested negative for point mutations in *SLC20A2*, *PDGFRB*, and *PDGFB*. The presence of copy number variations of *SLC20A2* was investigated by quantitative real-time PCR. Each of the eleven exons of *SLC20A2* was tested. A sample carrying a heterozygous deletion of all *SLC20A2* exons was included as a positive control. Quantification of *SLC20A2* revealed a large deletion encompassing exons six to ten in one patient from an Italian family. No additional alterations were found in the other screened individuals.

This result further underlines the importance of copy number variations in the etiology of PFBC. In conclusion, quantitative mutational screening of *SLC20A2* is warranted in affected families without pathogenic small sequence changes in known PFBC genes.

PS09.101

Expanding the PNKD mutation spectrum

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Paroxysmal non-kinesigenic dyskinesia (PNKD) is an autosomal dominant movement disorder characterised by attacks of dystonia, chorea and athetosis. To date, 3 mutations in the mitochondrial targeting sequence (MTS) of the PNKD gene account for all the cases in the literature. We describe a family in which the proband presented with a 'stroke-like' episode aged 43. Symptoms, including cognitive dysfunction and impaired motor function, subsided after 2 hours leaving the patient with a severe headache. Extensive investigations showed no evidence of a stroke. The patient's father has had over 50 episodes of weakness, labelled 'strokes', from a young age but without residual deficit. In light of this, screening for 'hemiplegic migraine' was requested. Sequencing using a next generation sequencing (NGS) panel of 11 genes encoding brain ion channel proteins detected a deletion of a C nucleotide in the last exon of the PNKD gene, c.1022delC p.(Pro341fs), in the patient and her father. This deletion results in a premature stop codon 3 amino acids downstream and a slightly truncated protein. While it is unlikely that the protein will undergo nonsense mediated mRNA decay (functional work is ongoing to confirm this), the function of the hydroxyacyl glutathione hydrolase domain may be disrupted leading to the build-up of the toxic metabolite methylglyoxal and related compounds which may reasonably be assumed to cause the phenotype. This case report expands the PKND mutation spectrum, resolves the diagnosis for this family and emphasises the usefulness of NGS in a diagnostic setting.

PM09.102

tRNA processing defects in pontocerebellar hypoplasia

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Introduction: Pontocerebellar hypoplasia (PCH) represents a heterogeneous group of neurodegenerative disorders with a prenatal onset. Symptoms are hypoplasia and/or atrophy of the pons and cerebellum and patients suffer from severe cognitive and motor defects. Aberrations in different tRNA processing genes, e.g. the tRNA splicing endonuclease (TSEN) genes and cleavage and polyadenylation factor 1 subunit 1 (CLP1), have been associated with PCH. However the disease mechanism remains elusive. Our research focuses on further revealing the link between PCH and tRNA processing. For this, we select genes of the tRNA processing pathway and assess their possible involvement in the PCH disease mechanism

Material and methods: Exome sequencing was performed in 25 PCH patients and 5 trios. Candidate genes were knocked down in zebrafish using morpholinos and resulting phenotypes were analyzed using ISH and TUNEL

assay.
Results: On the bases of its function we selected RNA 3'-terminal phosphate cyclase (RtcA) as an essential part of the tRNA processing pathway. Knockdown of RtcA results in microcephaly, aberrant movement and abnormal brain development in zebrafish. Exome sequencing revealed a homozygous missense mutation at the active site of RtcA in a PCH2 patient.
Conclusion: RtcA is possibly involved in the same pathway as other PCH associated genes and invigorates the involvement of the tRNA processing pathway.

PS09.103
Pontocerebellar hypoplasia type III caused by nonsense mutation in PCLO

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Pontocerebellar hypoplasia (PCH) is a group of disorders that share a common feature of an abnormally small cerebellum and pons. Involvement of the cerebrum, such as progressive microcephaly, is also common. It is highly heterogeneous, and eight subtypes on clinical and genetic basis have been known. PCH type III (PCH3) is one of the subtypes we reported in an Omani pedigree and mapped to chromosome 7q11-21. We have extended the study of this pedigree performing genetic analysis including genome-wide SNP genotyping, linkage analysis and whole-exome sequencing identifying a homozygous nonsense mutation in the gene Piccolo (PCLO) as the cause of PCH3. The variant was predicted to eliminate the PDZ and C2 domains in the C-terminus of the protein. PCLO is a component of the presynaptic cytoskeletal matrix. It localizes to the synaptic active zone, and is thought to be involved in regulation of presynaptic proteins and synaptic vesicles. Our findings suggest that PCLO is crucial for the development and survival of a wide range of neuronal types in the human brain.
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PM09.104
Mosaic uniparental disomy of chromosome 15q in an atypical PCH patient

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Pontocerebellar hypoplasia (PCH) describes a heterogeneous group of neurodegenerative disorders with a prenatal onset. Common features are hypoplasia/atrophy of the cerebellum and ventral pons, progressive microcephaly and severe mental and motor retardation. Ten subtypes (PCH1-10) are described based on differences in phenotype and/or genotype. The majority of the PCH genes are involved in RNA processing. In about 40% of patients a mutation in one of the PCH related genes is identified, in 60% the genetic cause remains unknown. One of the goals of our research is to identify new candidate genes involved in PCH. Here we describe an atypical PCH patient with a neuronal migration disorder. A SNP array and read number analysis of exome data was suggestive of a mosaic paternal uniparental disomy (UPD) of chromosome 15q24.2- 15qter, which was confirmed by single cell genotyping. 34% of cells were homozygous for the paternal allele in this region and 64% of cells were heterozygous. The supposed mechanism is a somatic recombination. Upon re-examination of the exome variants, a deleterious heterozygous mutation in the *POLG* gene was identified on the maternal allele. POLG is essential for the replication of mitochondrial DNA and homozygosity for this mutation is supposed to be lethal. This probably explains the absence of cell homozygous for the maternal 15q allele. Tentatively, we would like to hypothesize that this cell line existed but died at some point during fetal development, leading to the brain phenotype in this patient.

PS09.105
Familial porencephaly - clinical and neuroimaging manifestations in persons with COL4A1 gene mutation.

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Mutations in the COL4A1 gene have been found to cause autosomal-dominant porencephaly and leukoencephalopathy. Additionally infantile hemiplegia, migraines, seizures, intracerebral hemorrhage, ischaemic stroke, hematuria, arrhytmias may occur. We report here a family with a novel COL4A1 gene mutation and different clinical phenotype.
Our index patient is a 50-year-old woman, who has had migraine like headaches during last 5 years, hypertension since she was 30, microhematuria and unspecified eye damage since childhood. Last year she had transitorial ischaemic attack. Brain MRI showed left frontal horn porencephalic enlargement of the lateral ventricle, leukoencephalopathy and small T-2 hyperintensities in area of basal ganglia. A novel heterozygous mutation (c.1826G>A, p.Gly609Asp) was identified in COL4A1 gene. This mutation has not been described previously, but is predicted pathogenic because Gly substitution hampers the formation of the triple helix. Patient´s daughter (25y) had congenital hydronephrosis, no other symptoms and nonspecific MRI findings. Patient´s son (20y) had a subclinical stroke in perinatal period and an episode of supraventricular tachycardia at age 2 months. His physical and mental development is normal. His MRI is similar to mothers. He has arrhythmic episodes, frequent epistaxis and he becomes easily tired. Patient´s sister (30y) has had severe headaches during last 10 years. Patient´s mother died from cerebral infarction at age 69. Their genotypes have not been investigated.
It is noteworthy that our patient´s phenotype is quite mild and her daughter has no complaints, however she carries the same mutation. Consequently clinical picture among COL4A1 gene mutation carriers can be very variable.

PM09.106
Report on two families with inherited PTEN associated white matter lesions and neuropsychiatric disease presentation

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Recently, white matter (WM) lesions have been identified in patients with PTEN hamartoma tumor syndrome (PHTS). It is unclear how/if these findings correlate with the neurocognitive features associated with PTEN mutations, such as autism spectrum disorder (ASD) or developmental delays. We report two families with PTEN mutations and WM changes on their brain magnetic resonance imaging (MRI). The first family presented with neuropsychiatric features, and the second with developmental delays in the child. All have macrocephaly and WM hyperintensities on their MRIs in varying degrees and localizations. The father in the first family has an adult onset intermittent movement disorder (other possible causes ruled out) and no reported developmental difficulties, with a remote history of bipolar disease. His MRI shows multifocal leukoencephalopathy with subcortical and periventricular confluent areas of signal abnormality. His daughter has obsessive compulsive disorder and a mild learning disability. Her MRI shows much smaller bilateral subcortical WM lesions. The father in the second family presented with macrocephaly and penile freckling, and has subcortical and periventricular WM lesions, in keeping with the WM lesions seen in patients with PTEN mutations. His son has motor delays and has similar periventricular changes on his MRI. Previous reports identified a static WM disease in children with PHTS and developmental delay. A correlation between the extent of WM changes, reduced PTEN expression and cognitive function in ASD has been proposed. Clinical features in our patients with PHTS suggest an expanded PTEN phenotype spectrum, including previously unreported neuropsychiatric disease with possible progression in some individuals.

PS09.107
Pyridoxine-dependent epilepsy in Bulgarian dizygotic twins: a novel mutation in the ALDH7A1 gene

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Pyridoxine-dependent epilepsy (PDE, MIM 266100) is rare autosomal recessive disorder clinically characterized by a combination of various seizure types. The seizures usually present in the first hours of life and can not be

controlled with conventional antiepileptic medications but respond both clinically and electrographically to large daily supplements of pyridoxine (vitamin B_6). The dependence is permanent, and the interruption of daily pyridoxine supplementation leads to recurrence of seizures. The disease is caused by mutations in the *ALDH7A1* gene (MIM 107323) which is localized at 5q23.2 and encodes antiquitin, an alpha-aminoadipic semialdehyde dehydrogenase.

Here we report a dizygotic twin pair with classical form of PDE. Two hours after birth the infants presented with multifocal clonic seizures, resistant to anticonvulsive therapy. Direct sequencing of the *ALDH7A1* gene revealed one novel (c.297delG, p.Trp99*) and one already reported (c.328C>T, p.Arg110*) mutation. This represents the first genetically proven PDE case in Bulgaria. Our study enriches the spectrum of observed *ALDH7A1* mutations presenting a case with one novel single nucleotide deletion, located in exon 3 outside this "hotspot" region of the *ALDH7A1* gene containing ~60% of the reported mutations.

We emphasize that in patients with clinical diagnosis of PDE, even from Caucasian origin, it is necessary to examine the whole gene in order to confirm the diagnosis on molecular level. The increased number of described *ALDH7A1* mutations and genetically proven cases would help determining the real PDE frequency.

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PM09.108

Network analysis of Parkinson's disease genes identifies a novel function of RAB39B

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Gene coexpression network analysis assumes no prior biological knowledge, making it a very powerful tool for the identification of novel pathways and gene interactions. In Parkinson's disease (PD) more than 40 genes and loci have been identified so far, but how their disruption leads to a single clinically identifiable disorder remains to be elucidated. As part of the UKBEC project, we used Weighted Gene Coexpression Network Analysis (WGCNA) to explore the brain transcriptomes of 101 neuropathologically normal individuals to investigate the biological relationships between known PD genes and PD-associated loci. In substantia nigra, the region most affected by PD pathogenesis, we identified two modules; one significantly enriched for Gene Ontology terms related to mitochondrion and oxidative phosphorylation, and the other for synapse related terms. We then assessed the predictive power of this analysis by using the network constructions to investigate the function of RAB39B, a recently identified cause of X-linked intellectual disability and early onset Parkinson's disease and a member of Rab family of proteins known to be important for regulation of vesicular trafficking. As predicted by RAB39B's membership within the module related to oxidative phosphorylation, we show that RAB39B disruption in vitro leads to mitochondrial complex 1 inhibition and decrease in mitochondrial membrane potential supporting the role of RAB39B in regulation of mitochondrial function. Our work validates the use of gene coexpression networks to predict novel biological functions, and provides new insights into molecular pathways and interactions of PD genes and loci.

PS09.109

Genetic diagnosis of Rett syndrome by New Generation Sequencing: our experience.

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Rett syndrome (RTT) is an early-onset neurological disease that almost exclusively affects girls and is totally disabling. The RTT has a prevalence of 1/15000 being responsible for 10 % of severe mental retardation in females.

The clinical diagnosis is essential, since the discovery of a mutation confirms the diagnosis, but does not necessarily establish. Several genes that cause disease had been described: MECP2, CDKL5 and FOXG1.

We present a genetic study of patients using RTT -like New generation sequencing (NGS) .

We have designed a gene panel of 17 genes related to the RTT -like clinic presentation by HaloPlex Target technology. Enrichment System, for Illumina Sequencing. Sanger sequencing was used in exon not well covered and MLPA was done by causative RTT genes (MECP2, CDKL5 and FOXG1).

We studied patients with clinical RTT without genetic diagnosis and RTT patients with negative results by Sanger and MLPA of MECP2, CDKL5 and FOXG1 genes.

NGS results have been verified by Sanger sequencing and studied the origin of the mutation in the parents.

We have detected mutations in RTT-like patients in RTT genes and genes that do not cause RTT pathology, but overlap some features of RTT patients.

The genetic study by NGS allows to study a larger number of genes associated with RTT simultaneously, significantly reducing response time and the cost of the study. It also allows us to study other related clinical RTT and thus to redirect the clinical diagnosis to another disease genes.

Verification by Sanger of the progenitors of the mutations detected by NGS remains essential for their characterization.

PM09.110

RNA-seq based co-expression networks on human substantia nigra and putamen

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The substantia nigra and putamen are key brain regions implicated in the pathogenesis of Parkinson's Disease, one of the most common neurodegenerative disorders. We used Weighted Gene Coexpression Network Analysis (WGCNA) to identify common and private gene modules in these brain regions.

As part of the UKBEC project, we analysed RNA-seq data from 134 neuropathologically normal controls to construct co-expression networks (signed correlation, soft threshold =11) separately for each tissue using gene level expression. Gene expression values (GC-corrected normalised RPKM) were corrected for batch effects and other biases using 9 PEER axes. We analysed ~16k Ensembl annotated genes for both brain regions, with per-gene QC filters of RPKM>0.05 and missingness<20%.

Our substantia nigra and putamen networks revealed 73 and 56 modules containing between 33-1316 and 33-1171 genes respectively. We studied enrichment with ToppGene and found enrichment for similar Gene Ontology terms in both tissues, including brain-related terms like neuronal signalling, development of nervous tissue, neurotransmitters, oligodendrocytes and glial cell formation. We also found enrichment for relevant pathways, including axon guidance, and for diseases including epilepsy, neuroticism, and substance related disorders.

We investigated the putative biological function of the 613 lincRNAs that were expressed in our data. We found that 18 and 30 were highly associated with 3 and 6 modules in the substantia nigra and putamen networks respectively, providing immediate hypotheses for their previously unknown regulatory function. Our results demonstrate the utility of performing RNA-seq based gene coexpression network analysis in the human brain.

PS09.111

SHANK3 variants confer risk for schizophrenia and indicate a genetic overlap with autism spectrum disorders

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The SHANKs are
postsynaptic scaffolding proteins at glutamatergic synapses in the brain that
are essential for proper synapse formation
and maintenance. The *SHANK* gene
family (comprising *SHANK1*, *SHANK2* and *SHANK3*) is linked to a spectrum of neurodevelopmental disorders,
including intellectual disability and autism spectrum disorders (ASD). Schizophrenia
(SCZ) is a neuropsychiatric disease with high variability in the clinical phenotype, characterized by major impairments in perception of reality

and
disorganized thought or behavior. Different studies have already pointed to an
impairment of glutamatergic synaptic plasticity as an underlying cause of SCZ
pathology. To elucidate a putative contribution of genetic *SHANK3* variants to the etiology of SCZ, we sequenced the gene in
500 affected individuals and compared the sequencing results to ancestrally
matched controls. Novel SHANK3 missense
variants were identified in 1.6 % of the screened individuals, three of which
were predicted as deleterious by at least two different algorithms. We identified association of 5 genetic variants, with study-wide significance (P<0.001). Combined with previous studies, the rare G>V variant was found
in 4 out of 1543 SCZ patients and in 4 out of 2147 individuals with autism spectrum disorders (ASD), but not in 9315 controls.
We conclude that the *SHANK3* gene harbors different genetic
variations predisposing to SCZ, ranging from common and uncommon variants to
rare deleterious missense mutations. The SHANK3-G>V variant was found in
both ASD and SCZ patients, pointing to an overlapping genetic contribution of *SHANK3* to both neuropsychiatric
disorders.

PM09.112
Study of genetic association of HLA-DQ2.5 with circulating IgG antibody to cow's milk casein in schizophrenia

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Introduction: An increase in anti-casein antibodies has been reported in schizophrenia. Genome-wide association (GWA) study suggested that there was a lower frequency of the HLA-DQ2.5 genotype in schizophrenia patients than control subjects. The present work was thus designed to investigate the relationship between the HLA-DQ2.5 genotype, schizophrenia and circulating IgG antibody to cow's milk casein.
Methods: A TaqMan genotyping protocol was applied to genotype HLA-DQ2.5 in a total of 169 patients with schizophrenia and 261 healthy control subjects. Plasma samples from these subjects were used to detect IgG antibodies against 3 linear peptide antigens derived from α-casein (CSN1S1) and κ-casein (CSN3-1 and CSN3-2) using an in-house enzyme-linked immunosorbent assay.
Results: There was no association found between schizophrenia and circulating IgG to these three antigens. However, we observed an increase in IgG to CSN3-1 (p=0.035) and a decrease in IgG to CSN3-2 (p=0.0086) in DQ2.5 carriers compared with non-DQ2.5 carriers in the control group but not in the patient group. In addition, the frequency of HLA-DQ2.5 genotype was significantly higher in healthy controls than schizophrenia patients.
Conclusions: This study suggests that circulating IgG to cow's milk casein is not associated with schizophrenia but its secretion may be genetically controlled by the HLA-DQ2.5 genotype.

PS09.113
Schizophrenia-associated SNPs proximal to neurotransmission genes impact cognitive functions in patients and controls.

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Schizophrenia (SZ) is characterised by positive, negative and cognitive symptoms. As SZ is highly heritable, research has focused on GWAS, the most recent of which identified 83 new regions of interest associated with SZ. The link with specific functions in SZ is currently unknown. To take these results forward, these need to be identified, be that at the level of protein, neural pathway or phenotype. To characterise the effect of specific SNPs on cognitive function, selection was carried out based on the following classifications 1) proximity of SNP to gene, 2) unique association of gene to SNP, and 3) gene involvement in neurotransmission, which is disrupted in SZ. This resulted in nine SNPs in close proximity to genes involved in glutamatergic neurotransmission (GRM3, GRIN2A, SRR, CLCN3), signalling (CACNB2, HCN1, RIMS1) and receptor genes (DRD2, CHRN). To assess the impact of each SNP on cognitive function, neuropsychological measures (Table 1) were analysed. Analyses indicate significant effects on measures of cognition in patients and controls. SNPs showed an effect on scores of tests of social cognition such as the IPSAQ, Reading the Mind in the Eyes, and the Hinting Task showing that risk variants located within/proximal to gene regions involved in neurotransmission have significant effects on cognitive functions. Future work will aim to further elucidate this in conjunction with impact on imaging measures. Outcomes from such investigations may point towards neurotransmission pathways contributing to the disorder.

Cognitive Tests Used in SNP Analysis

Measure	Test
IQ	WAIS Verbal IQ
	WAIS Performance IQ
	WAIS Full-scale IQ
Episodic Memory	WMS Logical Memory 1 and 2
	WMS Faces 1 and 2
	CANTAB Paired Associate Learning
Working Memory	WAIS Letter-Number Sequencing
	CANTAB Spatial Working Memory
Attention	Sustained Attention to Response Task
	CANTAB Intradimensional/Extradimensional Set Shifting
	Continuous Performance Task
Social Cognition	Internal, Personal, Situational Attributional Questionnaire
	Hint Task
	Reading the Mind in the Eyes

PM09.114
An immunological study of 3 schizophrenia susceptibility genes identified by GWA study

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Introduction: Schizophrenia is a highly heritable disease based on family adoption and twin studies. A number of genome wide association (GWA) studies have been published, which have identified hundreds of genetic loci associated with an increased risk of schizophrenia. Interestingly, a recent study suggests most schizophrenia associated genes were highly expressed in B-lymphocytes, suggesting that dysfunction of humoral immunity may be involved in the pathophysiology of the disease.
In this study, we looked at the immunological response against 3 different antigens encoded by schizophrenia associated genes identified by GWA studies, including those coding for transcription factor 4 (TCF4), tripartite motif containing 26 (TRIM26) and disrupted in schizophrenia 1 (DISC1).
Methods: We developed an in-house enzyme linked immunosorbent assay (ELISA) to measure circulating IgG against the above 3 antigens in 100 patients with schizophrenia and 119 control subjects.
Results: The student's T test suggests that there was no significant difference in antibody levels for TRIM26 and DISC1 between the patient group and the control group (P>0.05), while a significant increase in TCF4 antibody levels was found in patients with schizophrenia compared to control subjects (P<0.05).
Conclusions: This study suggests that an autoimmune component is likely to be involved, in which circulating IgG to TCF4 may play a role, in the development of schizophrenia.

PS09.115
The involvement of DISC1, GRIA2A, GAD2 genes polymorphic loci in schizophrenia

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Schizophrenia is a severe multifactorial disease with a morbid risk of 7.2 per 1,000 and is affected by genetic and environmental factors. The disruption of the glutamatergic and the GABAergic systems, neuroplasticity changes are considered as an essential component of the pathogenesis of schizophrenia.
The aim of the current study was to determine the association of two polymorphic loci rs821597, rs843979 of DISC1 gene, two polymorphic loci rs2236418, rs928197 of GAD2 gene and polymorphic locus rs4403097 of GRIA2A gene with the schizophrenia in a sample of 338 cases (50% Russians and 50% Tatars) and 350 controls (50% Russians and 50% Tatars) from Volga-Ural region of Russia. The genotyping of these polymorphic loci was carried out by PCR-RFLP. The Haploview 4.1 program was used to determine the pairwise linkage disequilibrium and haplotype analysis. Odds ratios (OR) with 95% confident intervals (CI) were calculated.
The GAD2*A/A genotype (OR=3,62; P=0,0009) and the GAD2*A allele (OR=3,11; P=0,002) of polymorphic locus rs2236418 of GAD2 gene were found to be a risk markers of schizophrenia in Tatars ethnic group. The haplotype GAD2*AT from block which constructed of two polymorphic loci

rs2236418, rs928197 of GAD2 gene was identified as the high risk marker of schizophrenia in Tatars ethnic group (OR=2,15; P=0,0001). However previously mentioned polymorphic loci rs821597, rs843979 of DISC1 gene, rs4403097 of GRIA2A gene, rs928197 of GAD2 gene were not associated with the schizophrenia in Tatars and Russian ethnic groups.

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PM09.116

Schizophrenia associated variants in cognition: a schizophrenia GWAS follow-up

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In 2014, the Psychiatric Genetics Consortium (PGC) reported a schizophrenia genome-wide association study (GWAS) identifying 128 single nucleotide polymorphisms (SNPs) at 108 independent loci as associated with schizophrenia. This study aims to examine these variants for association with cognition in an independent schizophrenia sample. Genotypes at 125 SNPs were combined with MATRICS Consensus Cognitive Battery scores in a regression analysis for 592 individuals with schizophrenia. The social cognition domain was excluded due to reliability difficulties. 40 SNPs demonstrated nominally significant association with one or more cognitive domains. 8 SNPs were associated with the composite cognition score. 4 SNPs were associated with other cognitive domains following correction for number of domains examined (table 1). This study suggests a role for previously identified schizophrenia associated variants in influencing cognition. We will be attempting to replicate this in further independent samples.

Table 1. SNPs and association with cognitive domains.

SNP	Chr	Gene	Domain	beta coefficient	p
rs6704641	2q33.1	Intron SATB2	Composite	0.227	0.01
rs6704641	2q33.1	Intron SATB2	Speed of Processing	0.237	0.001
rs2905426	19p13.11	Unknown	Composite	-0.219	0.012
rs2332700	14q.24.2	Intron RGS6	Composite	0.22	0.015
rs190065944	15q25.1	Intron CHRNA5	Composite	0.276	0.019
rs56873913	19q13.33	Intron PRRG2	Composite	0.245	0.02
rs56873913	19q13.33	Intron PRRG2	Attention and Vigilance	0.271	0.008
rs2973155	5q33.1	Unknown	Composite	-0.2	0.024
rs2007044	12p13.33	Intron CACNA1C	Composite	-0.173	0.027
rs12522290	5q33.2	Unknown	Composite	-0.222	0.035
rs7907645	10q24.32	Unknown	Speed of Processing	-0.502	0.005
rs35518360	4q24	Unknown	Attention and Vigilance	-0.368	0.004

PS09.117

Assessing disease relevant interactions in schizophrenia.

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Schizophrenia is a highly heritable disorder in which genetic factors account for ~80% of the variability in liability. Genome wide association studies (GWAS) have shown that there are potentially thousands of risk loci associated with the disease (Sullivan et al. 2003). Although it has been shown that risk for schizophrenia is partially explained by additive effects of top-ranking single SNPs, interaction between SNPs may help to explain additional heritability that contributes to risk above and beyond that explained by single polymorphisms (Hemani et al. 2014; Zuk et al. 2012).

The CLOZUK dataset (Hamshere et al. 2013) consists of 5,199 cases ascertained through facilitation with Novartis, the manufacturer of a proprietary form of clozapine (Clozaril), and consisted of individuals with treatment-resistant schizophrenia. 5,873Controls were drawn from the Welcome Trust Case Control Consortium 2 (WTCC2) and the UK National Blood Transfusion Service by Cardiff University. Genotyping was done on two different chips.

As comprehensive pair-wise SNP-SNP interaction analysis is time consuming and computationally intensive, LD pruning was used to reduce the number of SNPs involved in the analysis. All pair-wise interaction effect sizes were calculated separately for each chip using logistic regression and then combined by meta-analysis.

Although no single interaction survived Bonferroni correction, there was evidence for enrichment of disease-relevant interactions amongst genes most highly associated with schizophrenia. The effect was more pronounced for interactions at higher significance levels. Similar effects were originally observed in a smaller, independent dataset (International Schizophrenia Consortium, 2008).

PM09.118

Association of COMT and MTHFR genetic variation with symptoms of schizophrenia

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Background. Schizophrenia is a common, complex multifactorial psychiatric disorder with a lifetime incidence of 1-2%. Changes in functioning of dopaminergic metabolism and intracellular methylation which are controlled by COMT and MTHFR genes could be risk factors of overall schizophrenia.

Aims. To investigate the role of a COMT (rs4680) and MTHFR (rs1801133) gene polymorphisms in the etiology of schizophrenia according to the severity of symptoms (PANSS rating criteria), in the Belarusian population.

Materials and methods. 125 schizophrenia patients and 90 controls were studied. Patient inclusion criteria were: ICD-10 schizophrenia diagnosis, age 27-65 (46.9 ± 9.5) years.

Results. No differences in allele and genotype frequencies of both MTHFR and COMT loci between schizophrenia patients and controls were observed. The Mann-Whitney test has demonstrated an association of MTHFR T-allele with greater scores of conceptual disorganization (p=0.001), grandiosity (p=0.051), mannerisms and posturing (p=0.009) symptoms while COMT G-allele - with greater scores of blunted affect (p=0.023), poor rapport (p=0.019), lack of spontaneity and flow of conversation (p=0.037), stereotyped thinking (p=0.052), motor retardation (p=0.027), poor attention (p=0.003) symptoms for polymorphism in schizophrenia patients. Notably, we observed significant associations of rs1801133 MTHFR T-allele with increased summation of ratings of Positive Scale and rs4680 COMT G-allele with greater summation of ratings of Negative Scale (p=0.014), General Psychopathology Scale (p=0.024), PANSS Total score (p=0.024) for patients.

Conclusions. Our results have shown that rs1801133 MTHFR affects the intensity of positive syndrome. Polymorphism rs4680 COMT influences the severity of patient's negative syndrome and symptoms of General Psychopathology Scale in clinical characteristics of schizophrenia.

PS09.119

An intersection of retroviral infection/reactivation, epigenetics and genetics in schizophrenia

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Schizophrenia is a disorder that can present itself in a broad spectrum of phenotypic variability ranging from single mild episodes to a dire need of life long treatment in a mental institution with around the clock care. The underlining concept of this seems to be driven not only based on the environment or the physical and mental health status upon onset, but is essentially attenuated via retroviral infection and epigenetic alterations in the context of an as yet undefined genetic landscape. Past efforts were in part not holistic enough to elaborate on the vastness in the plasticity of this psychiatric disease. The combined analysis of dendritic cells with viral particles in the cerebrospinal fluid, miRNA expression pattern derived from CSF-derived microparticles, the methylation analysis and gene alterations will be presented to derive signalling pathways explaining inflammation and virus reactivation in the CSF of patients with schizophrenia. Multi-parametric analysis is introduced as a sub-classifier with potential impact not only on more specific diagnosis, but furthermore to merit patient specific treatment in the future. Potential here lies in the use of anti-viral and immune modulating compounds as well as novel therapeutics shifting epigenetic signatures.

PM09.120

Genome organization and instability in the schizophrenia brain

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It has been repeatedly noted that chromosome/genome instability and abnormalities do affect the schizophrenia brain (Yurov et al., 2001; 2008). Alternatively, specific changes of the genome organization should co-occur with genome instability suggesting the existence of a cellular (endo)phenotype. Here, genome organization at chromosomal level has been addressed by a set of molecular neurocytogenetic techniques. Interphase chromosome-specific multicolor banding (ICS-MCB) was used to analyze genome organization and instability in the schizophrenia brain. The evaluation of 12 samples of the diseased brain and 12 control samples has shown a striking

discrepancy between schizophrenia and unaffected counterparts. Chromosome arrangement in interphase nuclei of the schizophrenia brain was completely different from the unaffected brain. The diseased brain has exhibited 10-40% of cells affected by genome/chromosome instability manifested as aneuploidy and chromosome breakage of chromosome 1. Additionally, remaining cells have almost all demonstrated a phenotype specific for chromosome instability. More precisely, 23-48% of cells in the schizophrenia brain were featured by the chromosome 1 localization at the nuclear periphery in contrast to controls characterized by chromosome 1 positioning near the nucleolus. Since such nuclear organization characterizes cellular populations affected by chromosome instability, we have suggested that these changes are likely to mediate genome instability in the schizophrenia brain. Accordingly, intranuclear organization of chromosomes in brain cells seems to be a new (previously unrecognized) epigenetic mechanism for schizophrenia.
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PS09.121

SCN8A mutation associated with intrauterine tremor, arthrogryphosis multiplex congenita and severe respiratory distress

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A newborn girl presented with severe respiratory distress needing intubation, continuous coarse tremor, lung hypoplasia, dysmorphic features, bell-shaped thorax and arthrogryphosis. EEG was initially normal but pathological at age 2 weeks. Two cerebral MRIs were normal. EMG findings suggested lower motor neuron disease. Epilepsy progressed and was refractory to treatment. She died after extubation at age 4 weeks. The mother felt tremor from the second trimester and no other fetal movements, and tremor was also observed by ultrasound in gestation week 24.
Methods and results: SNP-array and tests for SMA, Schinzel-Gideon syndrome and patUPD14 were normal. Whole exome sequencing revealed a de novo novel missense variant in SCN8A: c.718A>G p.(Ile240Val). Missense mutations in this gene cause epileptic encephalopathy with intellectual disability. SCN8A encodes Nav1.6, the α-subunit of a voltage gated neuronal sodium channel. This variant is located in an intracellular loop in juxtaposition to a transmembrane segment that operates as a gate sensor. Notably, an Ile to Val substitution in an equivalent position within another transmembrane domain (highly similar structure) was reported in a boy with neonatal epileptic encephalopathy, multiple congenital anomalies and movement disorder (Vaher et al; J Child Neurol 2014).
Conclusion: We report the first data on prenatal onset of movement disorder, including severe respiratory distress, likely caused by a missense variant in SCN8A. Dysmorphic features and arthrogryphosis are probably secondary to fetal akinesia. Our data adds to previous literature extending the phenotypic spectrum of variants in this gene.

PM09.122

Identification of seizure genes from 748 probands with developmental disorders

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The Deciphering Developmental Disorders (DDD) study aims to advance diagnosis of developmental disorders in the United Kingdom and Ireland through applying the latest genetic diagnostic technologies to samples from probands and their parents (trios). Children with developmental disorders may exhibit a variety of clinical phenotypes. One of the most common phenotypes observed in probands in the DDD study is seizures.
To date, the DDD study has analysed 4295 trios, 748 (17.4%) present with seizures. We attempted to identify mutations in genes that contribute to seizures within the subset of probands with at least one subtype of seizures. We sequenced the exomes of the members of each trio and interrogated genetic variants from the exome for *de novo* mutations within the probands. We identified genes significantly enriched with *de novo* mutations. Mutations in many of these enriched genes are reported to have a causative role in seizures, however we identified one mutated gene not previously linked to seizures and consider this a potentially novel gene implicated in seizures. We present the findings of our analysis and the potentially novel gene implicated in seizures.

PS09.123

Additional case of SLC6A1 and SLC6A11 (3p25.3) deletion in an autistic child with intellectual disability and EEG anomalies

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Introduction: Recently, three cases of overlapping microdeletions 3p25.3 showing a consistent phenotype with developmental delay, epilepsy, poor speech and stereotypes have been described. We present a forth case: A seven year old girl with intellectual disability (ID), absent speech and stereotypic movements.
Case report: Family history and perinatal period were unremarkable. Overall growth parameters were normal. Global developmental delay was noticed before age twelve months, her language development stopped and autistic behaviour became more apparent. Seizures presented at age five years, with fever. On physical examination she had a normal head circumference, poor eye contact, bruxism and an unusual but not ataxic gait. No specific dysmorphic features were observed. Brain imaging and metabolic screen were normal. EEG showed slow wave activity while awake and a diffuse epileptic activity. She is free of seizures under lamotrigine. Genetic investigations included fragile X testing and sequencing of CDKL5, which were normal.
Results: Chromosome microarray (180K array CGH) detected a 1.09 Mb microdeletion at 3p25.3, including SLC6A1 and SLC6A11. These genes encode gamma-aminobutyric acid (GABA) transporters. Alterations to any of these two genes have not been previously associated with human disease. However, biochemical and animal study data support the plausibility of a link between these two genes and EEG anomalies and ID. Variants in other neurotransmitter transporter genes (eg SLC6A4) have been found to increase the risk for psychiatric disease such as obsessive-compulsive disorder and autism.
Conclusion: We provide further evidence of an association between deletions affecting neurotransmitter transporter genes and autism spectrum disorders.

PM09.124

NGS reveals new causes for spastic quadriplegia in Roma patients

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We report on findings obtained by NGS of 552 genes in a small group of Roma patients affected with complicated spastic quadriplegia. Recently the homozygosity for a founder mutation in the PDHX gene (p.R446*) among Roma ethnic group was found to account for around 60% of cases presenting early in life with metabolic acidosis, spastic paraplegia/ quadriplegia, developmental delay and epilepsy and screening for this mutation was suggested as first step in clinical workup. Patients were selected on the basis of clinical presentation. We used the TruSight Inherited diseases panel, Illumina and the MySeq sequencing system. The search for the disease-causing mutations focused on variants with a quality score ≥ 30 and coverage ≥ 20 ×, located outside of segmental duplications and simple repeats. The step-wise filtering criteria included: allele frequency ≤ 1% in the 1000Genomes or NHLBI Exome Sequencing projects; "deleteriousness" predictions (RadialSVM Pred scores > 0.83357), splice-site (± 15 nt), nonsense, non-stop, and small in-frame or frame-shift in/dels. Six patients of Roma descent who were negative for the p.R446* founder mutation in PDHX gene were included in the study. We found causative mutations in two of them: RNASEH2B, p.Ala177Thr homozygous and AP1S2, p.Tyr86Ter hemizygous. The mutation in AP1S2 is novel. Our results show that recessive monogenic diseases such as Aicardi Goutieres syndrome 2 and Mental retardation, X-linked syndromic 5 may manifest at an early age, resembling cerebral palsy. NGS not only shortens the time for establishment of a diagnosis, but may alter the clinical course, if it reveals disease for which treatment is possible.

PM09.126

Missense variants as a possible cause of GRID2-related spinocerebellar ataxia type 18

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GRID2 is a member of the ionotropic glutamate receptor family of excitatory neurotransmitter receptors. Very recently, GRID2 has been associated with autosomal recessive spinocerebellar ataxia type 18 (SCAR18, OMIM: 616204) that is characterized by early onset severe cerebellar ataxia, ocular movement abnormalities, and intellectual disability with progressive cerebellar atrophy. So far, only in three families homozygous or compound heterozygous large deletions encompassing single or several exons of *GRID2* have been described as disease-causing. We performed whole exome sequencing (WES) in a Canadian patient and identified two *in trans* missense variants in the GRID2 gene: c.2128C>T (Arg170Trp) and c.2218G>A (p.Val740Ile). Based on ExAc, c.2128C>T was identified 6 times (MAF 0.00005) and c.2218G>A was identified 132 times (MAF 0.001, two homozygous cases). Both variants are located in highly conserved amino acid positions. Several, but not all, prediction programs suggested pathogenicity. qPCR experiments are ongoing to exclude large deletions/duplications. The patient has since the age of 8 months an early-onset episodic ataxia, failure to thrive, developmental delay, dystonic posturing and seizures. At the age of 12 years, the patient shows in addition progressive cerebellar atrophy. We see most symptoms of the patient clearly overlapping with those previously described in the three families with SCAR18. Although further characterization is needed, our results suggest that also missense variants, and not only deleterious deletions, might be disease-causing for SCAR18 with a comparable phenotype.

PM09.128

Dominance and recessiveness, two faces of the same coin? Illustration with two new genes in autosomal dominant spinocerebellar degenerations

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Introduction: Cerebellar ataxias (CA) and hereditary spastic paraplegias (HSP) are opposite ends of a wide spectrum of neurodegenerative disorders known as spinocerebellar degenerations. Approximately 200 causative genes have been identified, and transmission follows all classical modes. Therefore, next generation sequencing (NGS) is of great help in their molecular diagnosis, as well as for identifying new causative genes in unresolved families.

Methods: We used a combined approach of whole genome linkage analysis and exome sequencing in two families with either CA or HSP, aiming to identify the causative genes. Their involvement was validated with panel sequencing approaches, and functional studies when available.

Results: In a CA family, we identified a missense mutation in *GRID2*, heterozygous in late-onset affected adults, and homozygous in a child with congenital CA. In two patients with congenital CA, we identified *GRID2* mutations mimicking the *Lurcher* mouse model. In a family with HSP, a *ALDH18A1* missense mutation segregated with dramatically low plasma levels of citrulline, making it a potential trait biomarker. Four additional pedigrees, and in vitro analyses of ornithine metabolism, allowed establishing protein dysfunction.

Conclusion: Both *GRID2* and *ALDH18A1* had been implicated in autosomal recessive disorders in humans. With functional evidence, we established, in both cases, that AD transmission can occur, and is linked to a different phenotype. Aside from the novelty in the field of spinocerebellar degenerations, this illustrates the permanent landscape remodelling that occurs in human genetics since the arousal of NGS.

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PS09.129

An association between variants within the APOE gene and concussion profile: a preliminary investigation

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Introduction: Concussion is the transmission of biomechanical forces to the head resulting in neurological deficits, stimulating elevated release of brain tissue damage markers and promoting neural cell death. Apolipoprotein E is involved in nerve tissue integrity and encoded by the *APOE* gene. The ε4 allelic variant of the *APOE* gene was associated with greater severity for brain injury in boxers. Therefore, this study's aim was to investigate the association between *APOE* variants and concussion profile in rugby players.

Materials and Methods: In this case-control genetic association study, 198 previously concussed cases and 129 non-concussed, sport-matched controls (age: 15 - 39 years old) were recruited from rugby playing schools and clubs. Participants were genotyped for rs405509 (G/T), rs429358 (ε3/ε4) and rs7412 (ε2/ε3) variants within the *APOE* gene. Sport, concussion and medical history were recorded. Inferred haplotype analyses were conducted.

Results: No significant genotype or allele frequency differences were noted between cases and controls (>P=0.149) and for concussion symptom duration in cases (>P=0.253). However, the frequencies of the age-adjusted inferred G-ε4-ε3 (28% vs. 24%; P<0.001) and G-ε3-ε2 (25% vs. 19%; P=0.001) haplotypes, of the *APOE* variants (rs405509-rs429358-rs7412), were significantly greater among cases compared to controls, respectively.

Conclusion: Although the independent *APOE* genotype frequencies were not associated with concussion susceptibility or severity, the combined effect of the three *APOE* variants implicates *APOE* in concussion susceptibility. Further exploration is needed to understand the complex role of *APOE* in susceptibility.

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PM09.130

Mosaic dominant TUBB4A mutation in a highly consanguineous family with complicated hereditary spastic paraplegia

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Background: Mutations in TUBB4A have been recently associated with a spectrum of autosomal dominant neurological conditions, ranging from the severe hypomyelination with atrophy of the basal ganglia and cerebellum syndrome (H-ABC) to the clinically milder dystonia type 4 (DYT4). The presence of extrapyramidal symptoms was initially considered the only common hallmark of these disorders.

Methods: Clinical, neurological and neuroimaging examinations, followed by whole exome sequencing and mutation analysis were performed in a highly consanguineous pedigree with five affected children.

Results: We identified a novel c.568C>T (p.H190Y) TUBB4A mutation in a highly consanguineous pedigree mimicking autosomal recessive inheritance. The mutation have originated de novo in the mosaic mother of five children affected by complicated hereditary spastic paraplegia (HSP). The patients presented with an early onset, slowly progressive spastic paraparesis of the lower limbs, ataxia and brain hypomyelination, in the absence of dystonia or rigidity.

Conclusions: Our study adds complicated hereditary spastic paraplegia to the clinical spectrum of TUBB4A-associated neurological disorders. We establish genotype-phenotype correlations with mutations located in the same region in the tertiary structure of the protein.

PS09.131

TOSCA: What will it add to our knowledge of genotype-phenotype correlations in tuberous sclerosis complex?

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Tuber**O**us **SC**lerosis registry to increase disease **A**wareness (TOSCA), a multicenter, international disease registry, to study the manifestations, interventions, and outcomes in patients with tuberous sclerosis complex (TSC), has enrolled >2000 patients. The genetic research project of TOSCA, which is limited to European countries, aims to improve our understanding of genotype/phenotype correlations in TSC. TSC is most often attributed to *TSC1/TSC2* mutations, but limited genotype/phenotype correlations have been described so far due to the large number of mutations and the relatively small sample size of studies. Nevertheless, it has been shown that patients with *TSC2* mutation tend to be more severely affected, but with significant clinical overlap between individuals with *TSC1* and *TSC2* mutations, to the extent that it is inadvisable to make a clinical judgment based on an individual's mutation type. At first interim analysis of TOSCA, molecular testing had been performed on 301 of 508 (59.3%) patients. Pathogenic mutations were reported in 224 (74.4%) patients. Median time from clinical diagnosis to molecular testing was 30 months (range, 0-655). Overall, 125 (24.6%) patients had ≥1 relative diagnosed with TSC. TSC was diagnosed prenatally in 27 patients (5.3%; ultrasound finding in 19; family history in 8). As previously described in the literature, some features of TSC appeared to be more common in patients with *TSC2* mutations (ie, subependymal giant cell astrocytoma, infantile spasms, and renal angiomyolipoma). Given the larger size of the TOSCA cohort, quantitative analysis of more subtle and in-depth genotype/phenotype correlations will be performed, with the hope of improving the clinical utility of such findings.

PM09.132
Exome sequencing of a patient with Corpus Callosum agenesis

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Background
A 1 year old boy was referred to genetic counselling with an unresolved developmental disorder and corpus callosum agenesis. Previous genetic testing revealed a 245 kb deletion on chromosome 7p22.2 which was assumed to be of no significance as it was maternally inherited. MR caput revealed agenesis of the corpus callosum, whereas MR SPECT and EEG were normal.
Material and Methods
Exome trio sequencing was performed of the patient and his parents.
Results
We identified two heterozygous mutations in the EPG5 gene. The first mutation was a missense mutation inherited maternally and the second mutation was a frame-shift duplication inherited paternally. Both mutations were novel. Recessive mutations in *EPG5* are known to cause the rare Vici syndrome characterized by corpus callosum agenesis, hypopigmentation, cardiomyopathy, immunodeficiency and cataracts. Of these characteristics, only corpus callosum agenesis was present in this patient.
Conclusion
Although the patient does not fulfill the clinical criteria for Vici syndrome, we believe the two novel heterozygous mutations are the likely cause for the corpus callosum agenesis. Apparently he has a milder phenotype than previously reported in literature.
EPG5 mutation analysis may be of importance in other patients that presents with corpus callosum agenesis without other features.

PS09.133
Whole exome sequencing improves the diagnosis yield in sporadic infantile spasm syndrome

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Infantile spasms syndrome (ISs) is an epileptic encephalopathy characterized by clinical spasms with ictal electrodecrement, usually occurring before the age of 1 year and frequently associated with cognitive impairment. Etiologies of ISs are widely heterogeneous and an increasing number of genetic causes have been reported during the last years. However, the etiological diagnosis remains unknown in up to 40% of patients. We searched for de novo mutations in 10 ISs probands and their unaffected parents using WES. Inclusion criteria were the lack of consanguinity and of family history of epilepsy, as well as exclusion of common causes of ISs by brain MRI, metabolic screening, array-CGH and screening for mutations in CDKL5 and STXBP1, and for ARX duplications in males. We found a probably pathogenic de novo mutation in four patients. Two of them were missense mutations in SCN2A and in KCNQ2, in two patients who had no history of epilepsy prior to the onset of ISs. The third mutation, found in a female patient, involved, ALG13, located on the X chromosome. This mutation has been recently reported in three unrelated females with ISs. The fourth mutation was a three base-pair in-frame deletion in NR2F1, a gene whose mutations have been shown to cause intellectual disability and optic atrophy. In this study, we showed that WES can significantly improve the diagnosis yield in patients with sporadic ISs.

PM09.134
Unexpected molecular genetic findings by Next-Generation-Sequencing in a young ataxia patient

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Introduction: An 18 year-old patient of non-consanguineous parents had ataxic symptoms since age 16. The symptoms in the once excellent student were accompanied by progressive dementia, psychiatric changes, ocular apraxia, vertical ophthalmoparesis and non-specific elevations of liver enzymes, lactate and CK. He has a similarly-affected younger brother. His maternal grandfather had psychiatric issues, his mother's brother suffers from schizophrenia, autism, hypotonia and gait disturbance.Materials and Methods: The patient was evaluated for Niemann-Picktype C disease and SCA17 but no mutation could be identified. His DNA was then subjected to Next Generation Sequencing of 128 ataxia-associated genes and the results worked up clinically and segregated in the brother and mother.Results: The patient has a heterozygous SYNE1 variant once associated with autosomal dominant Emery Dreyfuss Muscular Dystrophy (EDMD4) as well as heterozygous mutations in WFS1 and PEX7, and a likely pathogenic variant in VPS13A. Of those, only the WFS1 mutation was also present in his brother. All mutations had been inherited through the mother. VPS13A alone has not been segregated. The genetic diagnosis of EDMD4 was not confirmed by muscle biopsy. In the course of disease his brother developed diabetes insipidus and signs of celiac disease. Both brothers have deteriorating vision that could indicate optic atrophy (ophthalmologic exam is pending).
Conclusion: The clinical presentation is now in line with Wolfram syndrome but a second mutation is warranted. We are currently establishing a microarray covering SNPs of ataxia-related genes. This may reveal a second WFS1-mutation in the two brothers and their father.

PS09.135
Diagnosis and management of Romanian children with Williams-Beuren syndrome

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DIAGNOSIS AND MANAGEMENT OF ROMANIAN CHILDREN WITH WILLIAMS-BEUREN SYNDROME (WBS)

OBJECTIVE: The present study focuses on the evaluation of the clinical characteristics of pediatric patients with WBS, to design a personalized management plan, to prevent the complications and to improve the prognostic.

MATERIAL AND METHODS: 22 children clinically and genetically diagnosed with WBS. The clinical diagnosis was based on the WBS diagnostic scoring system; FISH tests were performed to confirm the diagnostic.

RESULTS: All children were refered to our department for intellectual disability; 35% of them associated motor delay and 48%, poor language development. All children presented specific facial dysmorphism. Most children had particular behavior: temporal-spatial disorientation, hyperacusis, and attraction to music (100%); hypersociability (65%); hyperkinesias (35%); fussy eating patterns (12%). 17% of the children had cardiovascular disorders and 6% presented hypercalcemia. Some children presented atypical features: 2 children had autism and 1 child associated Marfan syndrome. 29% of patients were enrolled in physical therapy, and 42%, in different types of cognitive and behavioral therapy. Most of these children improved in their psychomotor development, well-integrating in regular education. 12% of the children were not enrolled in any therapeutic program and presented very slow psychomotor aquisitions, thus preventing their enrollment in any type of education.

CONCLUSIONS: Most of our patients presented the classical picture of WBS, but some children had different particularities which contributed to a delayed diagnosis. The management of WBS patients requires a multidisciplinary approach, and is essential for a better social integration of these children.

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PM09.136

Homozygous deletion involving WWOX confirms causality in early Infantile Epileptic Encephalopathy and allows for extensive detailed phenotyping in large consanguineous families

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WWOX (OMIM #605131) encodes WW domain-containing oxoreductase and acts as a tumour suppressor with a role in esophageal squamous cell carcinogenesis. A very recent association with early Infantile Epileptic Encephalopathy (IEE) has been reported, initially in a single child (of consanguineous parents) with a homozygous nonsense mutation and subsequently in eight individuals from two families with a clinical diagnosis of spinocerebellar ataxia type 12 and in two siblings with IEE, with compound heterozygous deletions and/or nonsense mutations (Mignot, 2015).

We present a very large consanguineous family with eight living and one deceased infant with undiagnosed encephalopathy investigated by array-CGH. A ~440kb homozygous deletion was identified at 16q23.1(77.75-78.19Mb). Homozygous deletions segregated entirely with affected children. Healthy siblings and parents were either heterozygous or normal. The deletion contains four RefSeq genes (NUDT7, VAT1L, CLEC3A and exons 1-4 of WWOX). Collation of our cohort and those published to date suggest an emerging pattern of poor postnatal growth, microcephaly and onset of seizures in the first year of life. Visual impairment and hypotonia are also observed, together with multiple deaths, mostly related to epilepsy. Specific genotype-phenotype granularities, potentially related to differing mutational mechanism / CNV gene content are presented.

IEE has heterogeneous causes including many single gene disorders that can be inherited or de novo; most are impossible to distinguish from each other on clinical grounds alone and the identification of WWOX reiterates the essential movement towards whole genome SNV and CNV analysis as prerequisite in clinical assessment.

PS09.137

Early onset epileptic encephalopathy in a male patient carrying an Xp11.23 deletion including the WDR45 gene.

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Mutations in the WD repeat 45 (WDR45) gene been have recently identified in patients suffering from neurodegeneration with brain iron accumulation (NBIA). NBIA is a genetically and phenotypically heterogeneous condition. WDR45 mutations cause a childhood onset encephalopathy accompanied by neurodegeneration in adulthood and iron accumulation in the basal ganglia. WDR45 mutations have been almost exclusively found in females and male lethality was suggested. Here, we describe a male patient suffering from a severe and early neurological phenotype, initially presenting as neonatal stormy seizures associated with an altered interictal EEG pattern and abnormal neurological development. This patient is a carrier of a microdeletion of chromosome Xp11.23 containing three genes, including WDR45. These findings reveal that deletions of WDR45 are viable in males and can initially be diagnosed as early onset epileptic encephalopathy without brain iron accumulation.

PM09.138

New insights into the genetics of X-linked dystonia-parkinsonism (XDP, DYT3)

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X-linked recessive dystonia-parkinsonism is a rare movement disorder that is highly prevalent in Panay Island in the Philippines. Earlier studies identified seven different genetic alterations within a 427-kb disease locus on the X chromosome; however, the exact disease-causing variant among these is still not unequivocally determined. To further investigate the genetic cause of this disease, we sequenced all previously reported genetic alterations in 166 patients and 473 Filipino controls. Singly occurring variants in our ethnically matched controls would have allowed us to define these as polymorphisms, but none were found. Instead, we identified five patients carrying none of the disease-associated variants, and one male control carrying all of them. In parallel, we searched for novel single-nucleotide variants using next-generation sequencing. We did not identify any shared variants in coding regions of the X chromosome. However, by validating intergenic variants discovered via genome sequencing, we were able to define the boundaries of the disease-specific haplotype and narrow the disease locus to a 294-kb region that includes four known genes. Using microarray-based analyses, we ruled out the presence of disease-linked copy number variants within the implicated region. Finally, we utilized in-silico analysis and detected no strong evidence of regulatory regions surrounding the disease-associated variants. In conclusion, our finding of disease-specific variants occurring in complete linkage disequilibrium raises new insights and intriguing questions about the origin of the disease haplotype, the existence of phenocopies and of reduced penetrance, and the causative genetic alteration in XDP.

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PS10.01

Primary fibroblasts cultures reveal TDP-43 abnormalities in Amyotrophic Lateral Sclerosis patients with and without SOD1 mutations

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A landmark in Amyotrophic Lateral Sclerosis research was the discovery of TDP-43 as major component of the abnormal protein aggregates that form skein-like and spherical inclusions or appear as diffuse granular material in neuronal and glial cells. Additional studies have confirmed that TDP-43 plays a central role in the pathogenesis of ALS and related neurodegenerative conditions, including frontotemporal lobar degeneration with ubiquitin positive aggregates. Studies on ALS patients have consistently shown that, in addition to cytoplasm accumulation, TDP-43 undergoes post-translational modifications, including hyperphosphorylation, ubiquitination and cleava-

ge into small C terminal fragments (CTFs). In the present study we examined TDP-43 expression in primary fibroblasts cultures from 22 ALS patients, including cases with SOD1, TARDBP, FUS, C9ORF72 mutations and nine patients without genetic defects. By using a phosphorylation-independent antibody, fifteen patients showed notable alterations of TDP-43 level in the nuclear or cytoplasmic compartments. In particular, a marked accumulation of TDP-43 was observed in the cytoplasm of all cases with C9ORF72 and TARDBP mutations, in one patient with FUS mutation and in three patients without genetic defects. In patients with SOD1 mutations, TDP-43 was significantly reduced in the nuclei and it lacked cytoplasmic mislocalization. These changes were associated with the presence of truncated and phosphorylated TDP-43 species. Our findings show that fibroblasts from ALS patients recapitulate some of hallmark TDP-43 abnormalities observed in neuronal cells. Though TDP-43 appears to be differentially processed in fibroblasts versus neuronal cells from ALS patients, primary fibroblast cultures may represent a helpful tool to investigate TDP-43-mediated disease mechanisms.

PM10.02

A novel mutation in the *MYBPC1* gene causes atypical Arthrogryposis Multiplex

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Introduction

Arthrogryposis multiplex congenita (AMC) is characterized by nonprogressive multiple joint contractures appearing at birth. We present a 20-months-old boy, the third child of Israeli-Druze first-degree cousins. He presented with AMC, hypotonia, hearing impairment, FTT and mild motor delay. His older deceased sister was described with a similar but more severe phenotype, which included AMC, cleft palate, feeding problems and severe developmental delay. The extended family includes a 16 y/o cousin presenting with isolated AMC, whose deceased sister was described with AMC and cleft palate.

A variable intra-familial phenotype and expected autosomal recessive inheritance prompted molecular diagnosis by whole-exome sequencing.

Methods

Following informed consent by the parents, whole-exome sequencing was conducted on the child's genomic DNA. Variant analysis focused on rare homozygous changes, followed by validation of candidate variants via Sanger sequencing and segregation analysis.

Results

Exome analysis revealed a homozygous missense variant in the *MYBPC1* gene, NM_001254718.1:c.481G>A (p.E161K), affecting the last nucleotide of exon 6. This novel variant was not observed in the common variant databases and segregated as expected within the extended family.

Discussion

The *MYBPC1* gene encodes a slow skeletal muscle isoform, essential for muscle contraction. Heterozygous mutations in this gene are associated with distal arthrogryposis type 1b, whereas a homozygous nonsense mutation is implicated in lethal congenital contracture syndrome 4. Our family expands the phenotypic spectrum of *MYBPC1* mutations. Our novel c.481G>A variant alters the last nucleotide of the exon 6 and therefore may affect splicing, allowing residual protein function which leads to a milder phenotype.

PS10.03

Mutation screening in rare myopathies

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The myopathies are neuromuscular disorders in which the primary symptom is muscle weakness due to dysfunction of muscle fiber. Other symptoms of myopathy can include include muscle cramps, stiffness, and spasm. The aim of our study was to identify the underlying genetic abnormalities in case of two patients with rare myopathies. A 4-year-old Hungarian boy presenting with the clinical and histological findings of nemaline myopathy type 2, and a 5-month-old infant with the histological diagnosis of centronuclear myopathy were investigated. Peripheral blood samples were taken from the patients and genomic DNA was isolated. Direct sequencing of the NEB and MTM1 genes was performed. The mutation screening of the NEB gene revealed two previously unreported heterozygous mutations: a deletion (c.24527_24528delCT p.P8176fsX8179) in exon 174 and a duplication (c.24250_24253dupGTCA p.T8085fsX8100) in exon 171 in the DNA sample of the patient with nemalin myopathy. In case of the patient suffering from centronuclear myopathy, the genetic analysis detected a hemizygous nonsense mutation (c.1456C/T p.Arg486X CM990881) in the MTM1 gene. These mutations result in the formation of premature termination codons, thus tey presumably leading to truncated nebulin and myotubularin proteins. Our investigations have great importance for the affected families since they help family planning. Hopefully, these findings might also provide the basis of future studies for the development of novel therapeutic modalities in neurogenetic disorders.

PM10.04

Exome sequencing in a patient with Charcot-Marie-Tooth disease

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Background

Charcot-Marie-Tooth disease (CMT) is characterized by progressive loss of the peripheral motor and sensory nerves and is the most common inherited neuropathy, affecting 1 per 1,214 persons in the general population. More than 70 CMT genes have been identified. The duplication of peripheral myelin protein 22 (PMP22) causes 20-50% of all CMT cases while point mutations are assumed to cause the remaining CMT cases.

Genetic analysis to discover point mutations have traditionally been performed with the laborious and time consuming Sanger sequencing.

High-Throughput Sequencing (HTS) is fairly quick and to a low cost compared with old methods when sequencing many genes simultaneously. Exome sequencing is a technique for sequencing all the protein-coding genes in a genome.

Material and Methods

A sporadic CMT case and her parents were investigated with exome HTS sequencing.

Results

We identified a novel homozygous missense variant in the SET binding factor 1 (SBF1) gene probably causing CMT in this family.

Conclusion

To our knowledge, this is the second CMT family with SBF1 mutation.

PS10.05

Copy number variations in a population-based study of Charcot-Marie-Tooth disease

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Copy number variations (CNVs) are important in relation to diversity and evolution, but can sometimes cause disease. The most common genetic cause of the inherited peripheral neuropathy Charcot-Marie-Tooth disease is the *PMP22* duplication; otherwise, CNVs have been considered rare.

We investigated CNVs in a population-based sample of Charcot-Marie-Tooth (CMT) families. The 81 CMT families had previously been screened for the *PMP22* duplication and point mutations in 51 peripheral neuropathy genes, a genetic cause was identified in 37 CMT families (46%). Index patients from the 44 CMT families with an unknown genetic diagnosis were analysed by whole-genome array comparative genomic hybridization to investigate the entire genome for larger CNVs, and multiplex ligation-dependent probe amplification to detect smaller intragenomic CNVs in *MFN2* and *MPZ*.

One patient had the pathogenic *PMP22* duplication not detected by previous methods. Three patients had potentially pathogenic CNVs in the *CNTNAP2*, *LAMA2* or *SEMA5A*, i.e. genes related to neuromuscular or neurodevelopmental disease. Genotype and phenotype correlation indicated likely pathogenicity for the *LAMA2* CNV, the *CNTNAP2* and *SEMA5A* CNVs remained potentially pathogenic.

Except from the *PMP22* duplication, disease causing CNVs are rare but may cause CMT in about 1% (95% CI 0-7%) of the Norwegian CMT families.

PM10.06

Identification of causal genes in Congenital Myasthenic Syndrome by whole exome sequencing.

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Background
Congenital myasthenic syndromes (CMS) are a group of genetically heterogeneous disorders characterised by compromised function at the NMJ. CMS manifests in childhood with fatigable weakness of limb, ocular and bulbar muscles. There are ~20% patients with a clinical diagnosis that still remain genetically undiagnosed despite over 20 known CMS genes.
Aims
To identify novel CMS genes by whole exome sequencing (WES).
Methods
DNA from a cohort of patients with a clinical diagnosis of CMS was sent to deCODE genetics for WES. Variants in the exome were filtered to exclude those with a frequency greater than 1%, those unlikely to significantly impact the protein or not compatible with the inheritance model. Remaining variants were segregated within the families. Three potential candidate genes were identified in four families, which were then analysed *in vitro* and *in vivo* where possible. Zebrafish orthologues were identified for two of the genes, these were then knocked down via antisense morpholino oligonucleotide (MO) injection and phenotype investigated.
Results
MO injection in zebrafish for two genes caused abnormal movement in response to tactile stimulation, shortening and abnormal branching of motor axons, poorly defined somites and disorganisation of muscle fibres.
Conclusion
Three genes have been identified as possible causative genes in CMS by WES. The preliminary studies carried out on zebrafish demonstrated phenotypes consistent with CMS such as disorganisation of muscle architecture and abnormal branching of motor axons. To confirm results obtained using MOs, we plan to utilise CRISPR technology to target the genes in zebrafish.

PS10.07

A case of TTN-related autosomal recessive congenital centronuclear myopathy

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Gene TTN (titin; locus 2q31.2) is responsible for several phenotypes: cardiomyopathies and skeletal myopathies with or without heart involvement differing by inheritance, age of onset and other features. Centronuclear (myotubular) myopathy, in its turn, is clinically and genetically heterogeneous, MYF6-, DNM2-, BIN1-, MTM1-, CCDC78-related forms are known. Recently several cases of TTN-related recessive congenital centronuclear myopathy were described (Ceyhan-Birsoy et al, 2013). We present another case in 7-year-old boy, an only child in Russian family. In three weeks he underwent surgery on coarctation of aorta. By 1-2 years congenital myopathy became evident. Myopathy was predominantly proximal, with CPK 1000-1300 U/l, early knee and ankles contractures, kyphoscoliosis, pectus carinatum, feet deformation, mild facial dysmorphism and no cardiomyopathy. Mental development and brain MRI were normal. Muscle biopsy performed in five years detected centronuclear myopathy. DNM2 and MTM1 mutations were not found. By that time the boy sat independently and crawled actively. With further periodic treatment in China he was improving and recently began to walk with bent spine and self-support (hands on knees). In Beijing DEYI Clinical Laboratory exome sequencing was performed. Of numerous findings those in TTN were of probable diagnostic value: compound heterozygosity for mutations c.94123G>T (exon 358) and c.98868insA (exon 360) of paternal and maternal origin correspondingly were detected. We tested results by Sanger sequencing. Clarified TTN genotype was: c.[94120insA,94123G>T] + [98868insA]. The diagnosis permits prenatal/preimplantation DNA testing in the family. Coarctation of aorta in the child is evidently independent.

PM10.08

Mutation of SLC5A7 associated with distal hereditary motor neuropathy

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Neurodegenerative diseases are becoming increasingly prevalent with the aging population, and are among the major contributors to disability and disease worldwide. The identification of the gene defects responsible has played a major role in our understanding of the pathogenic processes involved, and provided opportunity to develop targeted treatment strategies.
The neuromuscular junction (NMJ) is a specialised synapse with a complex molecular architecture that provides for reliable transmission between the nerve terminal and muscle fibre. We previously identified a mutation in SLC5A7, which encodes the presynaptic choline transporter critical for normal NMJ signalling, as the cause of a dominantly-inherited motor neurone disease (distal hereditary motor neuropathy type VII; dHMN-VII). We established that the mutation responsible resulted in the dominant-negative interference of the mutant molecule with function of the wild type choline transporter, resulting in significantly reduced (although not completely abolished) transporter activity. Here, we investigated a second family with clinical features overlapping those of dHMN-VII. We identified a distinct frameshift *SLC5A7* mutation indicative of a similar dominant-negative disease mechanism. We also modelled the mutation identified in a cell-based system to investigate transporter function. Together our findings corroborate a dominant-negative disease mechanism arising from SLC5A7 mutation as a cause of dHMN-VII, and provide further insight into the role of aberrant choline transporter function in neurological disease.
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PS10.09

Algorithm of molecular genetic investigation in Duchenne muscular dystrophy (case report)

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Duchenne/Becker muscular dystrophy (DMD/BMD) is an X–linked neuromuscular disorder characterised by progressive muscular weakness. The incidence is 1 in 3500 male births.
We present an atypical case of a 6-year-old boy without family history, with a partial deletion of exon 51 of the DMD/BMD gene, which has not been described yet. We show the algorithm of investigation and methods used.
MLPA investigation performed at the age of 2 years has proved neither a deletion, nor a duplication. Sequencing of the whole gene revealed a possible partial deletion of exon 51. The range of this deletion was determined using aCGH. Primers located behind the deletion were selected and the resulting PCR product was sequenced, revealing the deletion of the last 60bp of exon 51 and a huge part of intron 51 (over 27kb). Thus the donor splice site is destroyed.
The investigation of the mRNA obtained from the muscle biopsy of the patient ascertained the occurrence of three transcription products, two of which changing the reading frame and subsequently leading to a premature stop codon, the third one lacking the last 46 codons of exon 51.
The diagnosis could have been determined thanks to the cooperation of several departments. A clinical geneticist and neurologist expressed suspicion of the disease based on elevated liver enzymes. The convincing clinical picture further developing led to deeper investigation and with the help of a molecular cytogeneticist and a pathologist to the precise diagnosis.
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PM10.10

Survival in patients with Duchenne muscular dystrophy and cardiomyopathy in long-term follow-up.

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Purpose: Duchenne muscular dystrophy (DMD) patients often have cardiomyopathy. Unsuccessful treatment of
congestive heart failure symptoms in DMD patients is a big problem.
Methods: in 68 patients follow-up was from 6 up to 18 years (all
with verified mutations of dystrophin gene). Cardiac examination was made.

Result: all the patients were found to have indications for the use of an ACE inhibitor and almost half of the children had those for the administration of beta-blocker. Patients were divided in two groups: group I (n=35) received long-term courses of an ACE inhibitor (captopril in a dose of 0,5 mg/kg/day)
and a beta-blocker (metoprolol by individually adjusting its dose). Despite recommendations, Group 2 (n=33) were not treated with the above drugs. Mortality
in group I during the follow-up period was 37% (13 patients), in group II - 60%
(20 patients). In group I mean death age was 21,15 years, in group II - 18,25 years (no significant difference were found). The part of survived patients to the age of 21 years was significantly higher in group I: related frequency 0,77 (95% CI 0,5983···0,94) versus 0,39 (95% CI 0,19···0,5974). Conclusion: early started long-term treatment by ACE
inhibitor
and a beta-blocker in patients with Duchenne muscular dystrophy significantly increased the part of survived patients to the age of 21 years.

PS10.11
Identification of a synonymous variant as disease causing mutation in a DMD carrier

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A fifty year old woman was referred to our laboratory for analysis of the dystrophin gene. Her brother had died from Duchenne muscular dystrophy with unknown molecular cause. Based on elevated CK-levels in 1988 she had been identified as probable DMD carrier. After exclusion of a large deletion/duplication sequencing of the *DMD* coding region revealed the novel heterozygous variant: c.1329C>T. The substitution is synonymous, but as it is located near the end of exon 11, an effect on splicing was suspected. In order to prove or exclude such an effect transcript analysis was essential. A muscle biopsy was not feasible and the possibility of creating a lymphoblastoid cell line was lacking. Therefore RNA extraction from buccal and nasal epithelial cells was attempted. Even though full length dystrophin is not expected to be expressed in these tissues, nasal epithelial cells, but not buccal cells, proved to be a good source of *DMD* mRNA for our purpose. An aberrant transcript not present in a normal control was identified, reamplified and sequenced. The aberrant transcript was found to lack the four last bases of exon 11, r.1328_1331delGCAA, thus leading to a frameshift and a premature stop codon, p.(Ser443Ilefs*5). The results were in accordance with several *in silico* analyses. Taken together our findings yield convincing evidence, that c.1329C>T indeed is a disease causing mutation. Furthermore, nasal epithelial cells, which in contrast to muscle biopsies and blood samples can be obtained in a non-invasive way, proved to be a good alternative material for transcript analysis.

PM10.12
Genetic confirmation of germline mosaicism in a Duchenne muscular dystrophy Tunisian family

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Introduction: Duchenne muscular dystrophy (DMD) is an X-linked recessive genetic dystrophinopathy related to the absence of Dystrophin cytoskeletal protein. This disease affects generally boys; women are vectors.
Objective: In this context, we report a molecular study in a Tunisian family affected by DMD. Genetic analysis showed a particular transmission profile indicating a maternal germline mosaicism.
Patients and methods: Here we report a family composed by four boys and two girls. Genetic study was performed by MLPA and a modified fluorescent PCR (TP-PCR). MLPA allowed deletions and duplications analysis of different dystrophin's gene exons. Genotyping of six microsatellite markers, using the TP-PCR technique, helped to establish the haplotype of each individual and to follow its transmission in this family.
Result: MLPA showed, in both affected boys, a deletion of the exons 61 and 62 of the Dystrophin gene. However, the genotyping study revealed the presence of the disease associated haplotype in three boys in whom one is phenotypically healthy with no deletion responsible for the disease.
Discussion and Conclusion: Genetic analysis showed a particular transmission profile indicating a maternal germline mosaicism. She transmitted the same haplotype of the X chromosome to three of her sons, of which only two are sick and carrying the deletion of exons 61 and 62 of the Dystrphin gene. This can be explained by the presence of two cell populations in the germ cells of the mother: one carries the deletion and the other not, allowing a random transmission of the disease to her sons.

PS10.13
MuSK - a new target for lethal fetal akinesia deformation sequence (FADS)

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Fetal akinesia is clinically and genetically heterogeneous disorders, with the common feature defined as reduced or loss of fetal movement. Several disease genes of fetal akinesia have been described, but the genetic etiology of majority of cases is still unknown.
We report on a family with recurrent fetal loss, where the parents had five affected fetuses with fetal akinesia deformation sequence (FADS [MIM 208150]) and one healthy child. The fetuses displayed no fetal movements from gestational age of 17 weeks, extended knee joints, flexed hips and elbows and clenched hands. There was polyhydramnion and no visible fetal stomach. Whole exome sequencing were performed on a family trio and only one candidate homozygous variant was identified in the fetus, c.40dupA (p.Thr14Asnfs*9), located in *MuSK* (muscle, skeletal, receptor tyrosine kinase). Segregation analysis revealed homozygosity for all affected fetuses, while the variant was not present in the healthy child. The c.40dupA variant leads to a frameshift in MuSK predicting a premature stop codon, but the mutated mRNA is not cleared by nonsense-mediated decay pathway, which is currently under investigation.
MuSK is an agrin-dependent receptor tyrosine kinase required for formation of the neuromuscular junction and missense mutations in this gene have previously been described in congenital myasthenic syndrome (CMS). Interestingly, MuSK is located in the same acetylcholine receptor pathway as several other genes reported to cause CMS and/or FADS.
Additional families with intra-uterine fetal death, fetal akinesia and FADS are under currently under investigation, aiming to increase knowledge of genetic etiology in unresolved cases.

PM10.14
GJB1 mutations in Croatian CMT patients

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Introduction: In heterogeneous group of hereditary motor and sensory CMT neuropathies, GJB1 mutations causing M.Charcot-Marie-Tooth X type 1 (CMTX1) are considered to be second most common, following PMP22 variants. The disease is most often characterized by severe distal muscles weakness and atrophy, sensory loss and foot deformity. Female carriers can be mildly affected or asymptomatic. The diagnosis is suspected on NCV profile, featuring an intermediate velocity range. We report the results of GJB1 clinical and molecular study in the first series of Croatian CMT patients.
Objective: The detection and phenotypic characterization of GJB1 mutations in Croatian CMT patients.
Materials and methods: Following clinical and EMG examination, 22 suspected samples from 18 families, negative for PMP dupl/del, were analyzed for the mutations in GJB1 coding region. Applied Biosystems 3130xl Genetic analyzer and BigDye® Terminator v3.1 Cycle Sequencing Kit were used. The prediction of new variants pathogenicity was made with PolyPhen-2 software.
Results: GJB1 missense point mutations were found in 12 patients from 9 families (7 heterozygous, 5 hemizygous). In 3 unrelated families p.Val170Asp was found. In each of the remaining 6 families different mutations were found (p.Leu108Pro, p.Val13Met, p.Ser49Pro, p.Arg183His). Two mutations were not described previously: p.Phe141Ser (c.422T>C) and p.Val177Met (c.529G>A). Very severe phenotype was characteristic of novel p.Phe141Ser in hemizygous and also heterozygous state. Cardinal phenotypic features are compared.
Conclusions: GJB1 mutations were found in one half of CMTX1 suspected patients/families. In hemizygous and also heterozygous patients, phenotype is often severe, yet varies from mild/asymptomatic to devastating, with earliest distal sensory involvement.

PS10.15

GNE variants in a family with a complex neurological syndrome

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A consanguineous Pakistani family with a complex neurological syndrome including dystonia, pyramidal signs, limited speech, and hearing loss (Arif et al. Mov Disord 2011;26:2279-2283) was investigated. An autosomal recessive mode of inheritance was assumed in the five affected siblings. The ancestors and two siblings were healthy. In order to identify the genetic cause, genome sequencing was performed in two patients. Filtering for rare, shared, homozygous variants, predicted to alter the protein sequence revealed variants in six different genes (FER1L6, ABP1, DIAPH3, DUSP5, EMG1, MPP2). Since none of the variants segregated within the family, filtering for compound heterozygous variants identified eight candidate genes (SQLE, SPRED1, GNE, LEFTY1, MYH9, CDC42BPA, DHX57, STAB1). Only the two variants in GNE (p.R101C, p.L332F [NP_005467]) segregated with the disease in the family. While L332F was novel and not found in ethnically matched controls, R101C had a minor allele frequency of 0.3% in 190 Pakistani controls and of 0.002% among Europeans at http://exac.broadinstitute.org/gene/ENSG00000164116. Both variants are in-silico predicted to be pathogenic and are located in the epimerase domain of Glucosamine (UDP-N-acetyl)-2-epimerase/N-acetylmannosamine kinase (GNE). GNE encodes a bifunctional enzyme needed for the rate-limiting step in sialic acid synthesis. Experiments to access epimerase activity in the mutants are underway. Mutations in GNE are known to cause GNE-related myopathy and sialuria. A disease-causing effect of our GNE variants might suggest pleiotropy for mutations in GNE. However, results are based on a single family only. Thus, further research is warranted to elucidate the full phenotypic spectrum of GNE mutations.

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PM10.16

Results of multigene panel testing for inherited peripheral neuropathy reveal a high prevalence of autosomal recessive genetic aetiology, not restricted to severe early onset disease

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Inherited peripheral neuropathy (IPN) is a clinically and genetically heterogeneous group of disorders involving more than 90 genes and various modes of inheritance. Since July 2013 we have provided a 56-gene IPN NGS panel assay as a specialist UK Genetic Testing Network service. This is a frontline diagnostic tool and has largely replaced single-gene Sanger sequencing. Of the 56 genes included, 26 are associated with an autosomal recessive form of the condition, and for the majority of those no diagnostic service was previously available.

During the first 18 months more than 500 patient results were issued following IPN gene panel testing. Of all the positive diagnostic results, up to one third involved recessive genes. We present the distribution of recessive cases, the genes and phenotypes involved, and highlight individual case studies.

Autosomal recessive inheritance might typically be expected in severe, early onset, progressive neuropathy; however in our cohort a large proportion of patients were over 18 upon genetic diagnosis, including some cases assumed to be sporadic. This broadens the spectrum of phenotypes associated with recessive genes.

Our data shows that gene panel testing is a powerful, unbiased approach for genetic diagnosis in IPN. It provides a higher diagnostic yield, faster and at a lower cost than sequential single-gene screening of the proband, allowing efficient clinical management and family follow-up.

PS10.17

IPS cells creation to generate iPSc-derived motor neurons: a new cellular model to progress in the understanding of the axonal Charcot-Marie-Tooth disease

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Induced pluripotent stem cells (iPSc) are a highly interesting solution to create and observe the behavior of specific and unattainable cells from a patient. Our team is interested in genetic peripheral nerves disease and in particular in Charcot-Marie-Tooth pathology (CMT). One of our objectives is the development of motoneurons models from patients using the iPSc strategy in order to better understand the pathophysiology of *GDAP1* gene. This gene was described in 1998 to be responsible for a CMT axonal form and it encodes a mitochondrial outer membrane protein, but its function is still unclear.

We first obtained dermal fibroblasts (DF) from skin biopsies of a healthy person and of a homozygous patient carrying *GDAP1* nonsense mutation. Then, we reprogrammed DFs into iPSc using non-integrative plasmids (Oct4, Sox2, Klf4 and cMyc). After amplification, all quality controls were performed to conclude that our iPSc had the same properties and capacities than embryonic stem cells and a normal karyotype. Finally, we optimized protocols to successfully differentiate these iPSc into rosettes (structures full of neural progenitors), then into neurons and finally into motoneurons for control and GDAP1 patients.

Generation of motoneurons using axonal CMT-patient-derived iPSc was a first crucial step to better understand the role of GDAP1 in this pathology. Dynamic study of these *in-vitro* motoneurons, like observing their mitochondria in real time, will be then possible. The endpoint of this study will be to perform screenings of various drugs, so as to restore the function of these cells.

PM10.18

Whole exome sequencing approach in genetic diagnosis of limb girdle muscular dystrophy.

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Limb-girdle muscular dystrophy (LGMD) is a heterogeneous group of genetically determined disorders, characterized by primary or predominantly proximal distribution of muscle weakness. So far over 25 different *loci* associated with the disease have been identified. However, a large overlap of phenotypic manifestations resulting from different gene mutations poses a challenge for determination of the exact type of the muscular disease. Moreover new genes are still to be discovered as up to 50% clinical LGMD cases are left without molecular diagnosis.

To accelerate molecular diagnosis and to find novel genes involved in the disease we have adapted Whole Exome Sequencing (WES) workflow. Variants prioritisation was based on predicted pathogenicity, dbSNP information, and affected molecular pathways.

So far we have analysed genomic DNA samples from 34 LGMD patients, and in 17 cases we found mutations in the genes already associated with the disease: *CAPN3*, *DYSF*, *FKRP*, *ANO5*, *PLEC1* and *SGCA*. Two patients had more than one known gene affected. Apart from causative mutations also additional variants in genes related to muscle diseases (*TTN*, *DES*, *COL6A2*, *COL6A3*, *LDB3/ZASP*, *RYR1*, *LMNA*, *LARGE*, *NEB*, *FLNC*) that might contribute to the clinical phenotype were found.

Genetic diagnosis was possible more often in a group clinically determined as dysferlinopathies and calpainopathies with 14 out of 20 patients harbouring pathological variants in known genes. In the group of 14 patients with less typical phenotype instant genetic diagnosis was made only in three cases. Genetic etiology of patients without mutations in already known LGMD genes is being further investigated.

The research was supported by the KNOW-MMRC project (JPF).

PM10.20

LMNA related Congenital Muscular Dystrophy (L-CMD) - clinical review of patients diagnosed at the Newcastle MRC centre for Neuromuscular Diseases in the last 10 years.

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Lamin A/C related muscular dystrophies can present in adulthood as Limb-girdle muscular dystrophy 1B and an autosomal dominant form of Emery-Dreifuss muscular dystrophy, or at birth or in very early infancy. Because of the risk of a life threatening cardiac involvement early diagnosis is crucial.

We review the clinical presentation and medical history of a cohort of 10 patients with a laminopathy associated to an early onset muscular dystrophy

(L-CMD, Lamin-related Congenital Muscular Dystrophy). Age at last clinical assessment was 1 to 38 years (mean age 13 years).The severity of the muscular involvement is variable, presenting at birth with severe neonatal hypotonia or in early infancy with an abnormal gait. Contractures and rigid spine can be present at birth but most frequently develop during childhood. Onset of other complications is also variable: severe feeding difficulties were observed in two patients requiring PEG insertion at 4 and 8 years respectively; impaired respiratory function requiring non invasive nocturnal ventilation was observed in two patients at 4 and at 35 years. Symptomatic cardiac arrhythmias requiring defibrillator or pacemaker implantation were observed in two patients, and one patient had asymptomatic sinus tachycardia and diastolic dysfunction at 5 years.
Due to the variable severity of the phenotype an early clinical diagnosis can be challenging, and the interpretation of a novel genetic variants, which requires particular care due to the possible cardiac risk of family members, can even be more complex and requires active interaction between the clinician and the genetic laboratory.

PS10.21

Bilateral congenital lumbar hernias in a patient with central core disease - a case report

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Congenital lumbar hernias are rare malformations caused by defects in the development of the posterior abdominal wall. A known association exists with lumbocostovertebral syndrome, however other associated anomalies have been previously reported, including one case with arthrogryposis, suggesting an association with myopathy. We present an infant girl with bilateral congenital lumbar hernias, multiple joint contractures, decreased muscle bulk and symptoms of malignant hyperthermia. Molecular testing revealed an R4186C mutation in the ryanodine receptor 1 (RYR1) gene, known to be associated with central core disease. This is the first reported case of a child with congenital lumbar hernias and central core disease.
Stromal interaction molecule 1 (STIM1), which modulates calcium flow through RYR1, plays an important role in skeletal muscle differentiation. Mutations in STIM1 have been previously shown to result in immunodeficiency and myopathy. It is possible that downstream RYR1 mutations may also impair store-operated calcium entry, leading to altered calcium-dependent signalling pathways that interrupt muscle differentiation and development. This case suggests an expansion of the central core disease phenotype to include congenital lumbar hernias.

PM10.22

Genetic diagnosis of the Myotonic Dystrophies by Molecular Combing

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Myotonic Dystrophy type 1 and 2 (DM1, DM2) together represent the most common muscular dystrophies in adults with a frequency of 1:8000. Both diseases are associated with an expansion of a microsatellite repeat within the untranslated regions of two different genes, DMPK and ZNF9, respectively.
Currently, the gold standard for genetic DM diagnosis is Southern blotting by which the repeat sizes for DM1 can be determined accurately. This is not the case for DM2, due to the somatic instability of the expanded alleles. Therefore, we developed an alternative diagnostic technique for DM based on molecular combing in order to analyze both repeat loci in parallel and to quantify expanded DM2 alleles.
Molecular Combing is an *in situ* hybridization like technique where locus specific probes are hybridized to uniformly stretched genomic DNA. Different loci can be detected by individually designed probes. We designed a diagnostic protocol for assaying the DM1 and DM2 loci together in one approach. The two loci including the repeat itself are reliable detected by two different probe sets and repeat sizes can be measured directly with an accuracy of 0.8 kb (±0.4). For the first time, the Molecular Combing DM-test allows for a quantification of expanded DM2-alleles on single molecules. We demonstrate that there is a wide size range of repeats in the sample of an individual DM2 patient which is in accordance with previous speculations in the literature. In order to use this method in routine diagnosis, improvement of the detection limit is needed to distinguish normal alleles from short but pathogenic expansions.

PS10.23

Progressive muscular dystrophies child (about 24 case)

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Progressive muscular dystrophies child (about 24 case)

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Progressive muscular dystrophies, term proposed by Erb in 1884, form a set of neuromuscular diseases of various etiologies and different severities. These are diseases characterized by progressive degeneration of muscle fibers with a histological formula necrosis refresh characteristic; they also show great phenotypic and genotypic heterogeneity.
In this present study, we were interested to take stock of the Sacroglycanoapthies due to their greater frequency in Morocco and their very close clinical characteristics, as well as the implementation of molecular diagnostics in the Laboratory Molecular Biology.
Based on the received clinical data, 24 patients with progressive muscular dystrophies were analyzed by PCR-SSCP screening SGCG the gene, more particularly by targeting c.del525T mutation in exon 6 of the gene, followed by sequencing of the disputed region.
Molecular tests we conducted showed that approximately 40% of patients had a deletion in the c.del525T SGCG gene in the homozygous state. This frequency clearly showed the molecular level the high frequency of these diseases in our country and stressed the importance of testing a priority in our patients.
The diagnostic approach that we used in our study seems well suited for driving in the first instance in our country, especially as it has the advantage of easily allowing a differential diagnosis.

PM10.24

MYH7 exon 31 mutations within the rod domain cause autosomal dominant muscular dystrophy in two un-related Jewish Moroccan families

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Skeletal myofibers are characterized by the expression of particular myosin isoforms. In slow twitch, type I fibers, which display oxidative metabolism and high endurance, the predominant myosin is MYH7, encoding the slow/β-cardiac MYH. MYH7 mutations have been associated with at least 5 different clinical phenotypes, among them are Dilated Cardiomyopathy 1S/ Left Ventricular Noncompaction 5 (MIM613426), Familial Hypertrophic Cardiomyopathy 1 (MIM192600), Laing Distal Myopathy (MIM160500), Myosin Storage Myopathy (MIM608358) and Scapuloperoneal Syndrome, Myopathic Type (MIM181430). Two unrelated families of Jewish Moroccan ancestry presented with apparently autosomal dominant heredity with partial penetrance of a unique progressive muscular dystrophy somewhat reminiscent of Laing Distal Myopathy. Whole exome sequencing identified in both families two different heterozygous mutations within exon 31 of MYH7: c.4309G>C (p.Ala1437Pro) and c.4301G>C (p.Arg1434Pro). Each of the mutations impair myosin self-assembly properties and has dominant negative activity in nonmuscle cells, as was demonstrated in COS-7 cells transfected with β-myosin constructs carrying the mutations. Of MYH7 mutations, proline mutations within the Rod domain were shown to cause Laing distal myopathy and to be the most damaging to MYH7 Rod domain. Thus, our functional studies demonstrate that the Laing-like partly-penetrant myopathy stems from mutations affecting the myosin rod domain.

PS10.25

Healthy, premutation and mutation range myotonic dystrophy type 2 alleles - seeking the approximate borders between stable and unstable repeat motifs

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Introduction: Myotonic dystrophy type 2 (DM2) is caused by expansion of a (CCTG)n repeat in the CNBP1 gene. In healthy-range alleles the repeat is generally interrupted by one or more GCTG, TCTG or ACTG motifs. Uninterrupted tracts were, however, described in expanded alleles as well as in larger healthy-range alleles which are generally considered as DM2 premutations. The threshold for pathogenicity is still poorly described in the literature, while the smallest reported pathogenic alleles had 55 and 75 CCTG repeats, respectively.

Materials and methods: For DNA analyses we used conventional PCR and bi-directional repeat-primed PCR complemented by Sanger sequencing and targeted massively parallel sequencing where needed.

Results: We identified wider range and higher frequency of uninterrupted alleles than it was previously reported, with 15 alleles characterised in total, spanning the whole spectrum of healthy-range alleles. We therefore further investigated the intergenerational stability of these alleles. Moreover, in two unrelated patients with symptoms of neuromuscular disorder we identified two ambiguous alleles, containing 31 and 34 uninterrupted CCTG repeats. Since further analyses revealed a full range DM1 expansion in the DMPK gene in the first patient and a homozygous CLCN1 stop mutation in the second patient we concluded that these "grey zone" alleles are most likely not pathogenic themselves, although, they represent unstable premutation alleles. Their possible modifying role, however, cannot be conclusively disclosed.

Conclusion: Our results suggest that uninterrupted alleles with up to ~30 CCTG repeats are likely stable during transmission, while instability gradually increases with increasing length of uninterrupted tracts above this approximate threshold (financial support: VEGA_2/0115/15).

PM10.26

A large deletion affecting *TPM3*, causing severe nemaline myopathy

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We present here the first large (17-21 kb) aberration in the α-tropomyosin$_{slow}$ gene (*TPM3*) identified using our self-designed nemaline myopathy (NM) CGH-array. The identified homozygous mutation deletes the promoter as well as the muscle-specific exons 1a and 2b of *TPM3*. The promoter and exon 1b of the non-muscle isoform seem to be intact. The homozygous deletion was identified also using whole-exome sequencing (WES). Previously, we identified one heterozygous nonsense mutation in *NEB* exon 42. We hypothesize that this nonsense mutation may have had a modifying impact on the severe phenotype of the patient. This female infant was born to first-cousin parents. Paucity of movement was noted from birth, and proximal and axial weakness was confirmed at 5 months. Best motor function achieved was rolling from side to side. Failure to thrive led to nasogastric tube feeding at ten and gastrostomy insertion at 13 months. The patient died at age 17.5 months. Muscle biopsy at 5 months showed marked variation in fibre size with several small fibres with slow myosin, some central nuclei, red-staining inclusions on trichrome stain in several fibres, which electron microscopy confirmed as rods, as well as central mitochondrial aggregates. We have screened 196 families with NM or a related disorder using the NM-CGH array. This *TPM3* deletion is the first large rearrangement outside the *NEB* gene, suggesting that large aberrations in the other NM genes are rare. They should, however, be taken into consideration. The NM-CGH microarray method is available for mutation analysis in our laboratory.

PS10.27

The importance of developing the Hellenic Neuromuscular Disorders (HNDR) Registry

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The Department of Medical Genetics of Athens University has been the reference centre for neuromuscular disorders in Greece since 1990, being the first lab to offer routine molecular diagnosis and prenatal testing for DMD/BMD (Duchenne / Becker Muscular Dystrophy), SMA (Spinal Muscular Atrophy) , Myotonic Dystrophy and FSHMD.

On 2012 the first national registry for neuromuscular disorders in Greece [Hellenic Neuromuscular Disorder Registry, HNDR)] was created, while on 2013 it has been connected with TREAT-NMD network. Currently the software is configured to register patients with DMD/BMD and SMA disorders and up to now we have included data on 155 patients, from which 18 have SMA and the rest DMD/BMD.

The HNDR has become a hub for information and awareness for the public and the means to link patients and their families with scientists in Greece and all over the world. This structure also enables patients to volunteer for research therapeutic protocols, opening the way for the creation of trial centers in our country. Beyond that, this database has been proven helpful in the research for neuromuscular disorders therapy, genotype-phenotype correlation, and population studies. The HNDR publicizes the extent of the problem in Greece, which additionally helps anyone interested in lobbying for the rights and needs of the patients with neuromuscular disorders.

PM10.28

Genetic testing of inherited muscular dystrophies and myopathies using Next Generation Sequencing

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The inherited muscular dystrophies and myopathies comprise a heterogeneous group of muscle diseases that share similar clinical features as progressive skeletal muscle weakness and wasting. Mutations in several genes that encode proteins of extracellular matrix, endoplasmic reticulum, nuclear envelope, sarcolemmal proteins and glycosyltransferases are known to be responsible for muscular dystrophies and myopathies. The large overlap of phenotypic manifestations resulting from different gene mutations poses a challenge for determination exact type muscular disease.

Fistly, we designed capture library to target the coding and all flanking intron regions of 42 genes associated with group of disease as muscular dystrophies, congenital muscular dystrophies, congenital myopathies, distal myopathies and other myopathies. We performed targeted capture combined with next-generation sequencing using NimbleGen SeqCap EZ Choice library in 140 Czech probands. Mutations associated with muscular dystrophies or myopathies were identified in 77 of them. For the rest of patients, we have to confirm detected variants and correlate them with clinical findings of patients. Mutations have been detected in *ACTA1, CAPN3, COL6A1, COL6A3, DMD, DNM2, DYSF, LAMA2,LMNA,RYR1, SGCG, SGCB* and *SEPN1* gene. To be able to offer complex diagnostics of neuromuscular disorders, we broaden our neuromuscular panel by adding of remaining genes from groups chosen in the first panel and by adding genes associated with other groups of diseases: myotonic syndromes, ion channel muscle diseases, metabolic myopathies, congenital myasthenic syndromes, motor neuron diseases and other neuromuscular disorders.

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PS10.29

A novel mutation in LRRK2 influences risk for Parkinson disease in the Maltese Population

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Introduction: LRRK2 encodes Leucine-rich repeat kinase 2, one of the most common known autosomal dominant genetic causes of Parkinson disease (PD). Individuals with mutations in LRRK2 present with a phenotype and pathology similar to idiopathic, late onset PD. A number of mutations have been identified in this gene, however, the pathogenic nature of most mutations remains unclear.

Materials and Methods: Next generation sequencing data from healthy individuals was mined for LRRK2 mutations present in the Maltese. A novel mutation (N618S) was identified and genotyped by PCR and RFLP in a PD case-control collection; samples from Malta collected as part of the Geoparkinson

project. Odds Ratio (OR) with 95% confidence interval (CI) was determined using logistic regression.
Results: The novel mutation identified was in exon 16 of LRRK2. The A>G change at c.1853 gives rise to a missense mutation: N618S. Preliminary analysis on part of the Maltese collection gave a minor allele frequency of 0.03 in controls (n=136) and 0.06 in cases (n=73), giving an OR of 2.17 (95%CI: 0.82 - 5.74).
Conclusion: The novel N618S mutation appears to increase risk for PD in the Maltese.
Funding sources for this study: Data and samples were collected as part of the 5th framework (FP5) EU funded Geoparkinson study, project number QLK4-CT-1999-01133. This work was supported by the MASTER it! Program (Malta); this scheme is co-funded by the ESF under Operational Program II-Cohesion Policy 2007-2013.

PM10.30
Genetic studies of patients with Parkinson's disease in Ukraine

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Parkinson's disease is the second most frequently occurring neurodegenerative disorder. The frequency of the disease in a population of people over 60 years is 2%. Diagnosis of the disease is very complicated at the young age, as the first symptoms appear, on average, at the age of 50-60 years. In this regard, especially promising are studies in the field of molecular genetics, results of which can be used in early diagnosis of the disease. The objective of this work was to find genetic markers for Parkinson's disease in populations in Ukraine. 350 patients with PD (210 men and 140 women) were examined after a mean disease duration of 7,6 ± 0,33 years (mean age was 63,1 ± 1,3 years).
Genotyping was performed by PCR-RFLP method and methods based on real-time PCR. For the first time in Ukraine, major mutations in the genes PARK2, LRRK2, SNCA and GBA responsible for hereditary forms of Parkinson's disease were genotyped. Frequencies of mutations in patients that had various peculiarities of the disease and were at different age of manifestation were analyzed. Comparison of telomere length in buccal cells and leukocytes of patients with PD and control group was carried out. Significant differences were revealed in the length of telomeres in buccal epithelium cells in patients and in controls, while in blood cells telomere length did not differ. In addition, a significant correlation between telomere length in blood cells and in buccal epithelial cells was found in patients, but not in control group.

PS10.31
Imbalance of excitatory/inhibitory synaptic expression in Rett syndrome iPSC-based neuronal models

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Introduction: Rett syndrome is a neurodevelopmental disorder due to de novo mutations in MECP2, CDKL5 or FOXG1 genes. MeCP2 and FoxG1 are transcriptional regulators; CDKL5 is a kinase involved in multiple cellular processes. In spite of their involvement in the same disease, a functional interaction between the three proteins has not been proven and disease mechanisms remain elusive. We reported recently that expression of GluD1 is increased in iPSCs-derived neurons from patients with mutations in *MECP2* and *CDKL5* (Livide et al, Eur J Hum Genet 2015). GluD1 is a post-synaptic protein that induces preferentially inhibitory presynaptic differentiation of cortical neurons.
Methods: We established a human neuronal model based on patients-specific iPSCs and we characterized their expression profile to test the hypothesis that common alterations occur in cells mutated in the three genes.
Results: We identified an over-expression of GluD1 also in FOXG1-mutated cells. Since GluD1 induces inhibitory presynaptic differentiation and an excitation/inhibition imbalance has been suggested in MECP2-related Rett syndrome, we tested the expression of a panel of excitatory and inhibitory markers. Our results demonstrated an excitation/inhibition imbalance in neurons mutated in all three genes. A consistent imbalance was also observed in embryonic FOXG1+/- mouse brain. However, a reduction of both excitatory and inhibitory markers is observed in post-natal mouse models.
Conclusions: Our data provide further evidence in favor of an excitation/inhibition imbalance as an important contributor to the pathogenesis of Rett due to MECP2 mutations and suggest a similar mechanism for CDKL5- and FOXG1-mutated patients.

PM10.32
Performance of SMN1 and SMN2 copy number assays using ddPCR

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Introduction: Spinal muscular atrophy (SMA) is the most common autosomal recessive disease causing death in infants. The disease is caused by the lack of SMN1. The copy number of a highly similar gene, SMN2, determines disease severity. It is difficult to measure SMN1 and SMN2 copy number accurately since these two genes only differ by a single functional nucleotide. Although no therapies currently exist for SMA, five different pharmaceutical companies have therapies in clinical trials. To assess the efficacy of these therapies, it is necessary to measure the number of SMN2 copies accurately in each trial participant.
Materials and Methods: 16 Coriell samples and 93 Kenyan, 70 Mexican, and 96 British HapMap samples were screened for SMN1 and SMN2 copy number using droplet digital PCR (ddPCR).
Results: 99.2% of the samples measured within 10% of the integer for SMN1 and SMN2 copy number, and 100% were within 15% of the integer. In total: 5, 1, 204, 45, and 20 samples had 0, 1, 2, 3, and 4 copies of SMN1, respectively. Likewise, 43, 109, 108, 14, and 1 samples had 0, 1, 2, 3, and 5 copies of SMN2, respectively. None of the 275 samples had 4 copies of SMN2.
Conclusions: ddPCR provides unambiguous copy number calls for SMN1 and SMN2 when ≤3 copies are present. We did not have sufficient number of samples with ≥4 copies to determine the accuracy of this technology for higher-order copy number states.

PS10.33
A comprehensive analysis of SMN2 target re-sequencing in a cohort of Italian and Spanish patients affected by Spinal Muscular atrophy.

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Homozygous deletion of *SMN1* results in a spinal muscular atrophy (SMA) phenotype. Severity of the disease partially relies on the individual copy numbers and variants of the phenotypic modulator *SMN2*. We used a NGS strategy of „pooled indexing" for gene re-sequencing of 41 SMA patients belonging from Italian and Spanish population. All subjects carry 3 copies of *SMN2*, 0 copies of *SMN1*. We report a total of 115 SNPs, among which 60 never previously identified, and 5 indels among which only one previously described. All indels and most of SNPs are intronic, except than two conservative SNPs in exon2a and exon3 respectively. The main intriguing results are: i. Variants distribution is not homogenous but rather clustered along the *SMN2* sequence. The highest variant rate have been found in intron 7 (1:112bp), in intron 8 and 3'UTR (1:152bp). These findings suggest that exon 7, which undergoes alternative splicing and is involved in modulating SMA phenotype, is flanked by hotspot of hyper-mutable regions. ii. Only two individuals present the exon2a variant (c.C84T), while 33 out of 41 individuals present the exon3 variant (c.A462G). It will be intriguing to test the last variant as an hallmark of deletion-prone chromosome 5. iii. In one patient we have a variants frequency sustaining 4 copies of *SMN2* along the 5' of the gene and 3 copies of *SMN2* along the 3'. This last result sustains quantitative re-sequencing of *SMN* as a tool to improve diagnosis/prognosis by identifying partially deleted and non-functional genes.
Grant references: POR FESR 2007-2013 " A platform for molecular and personalized medicine"

PM10.34
A natural variant (c.863G>T) in exon 7 of SMN1 disrupt the inclusion of Exon 7 into mRNA and is responsible for spinal muscular atrophy

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Proximal spinal muscular atrophy (SMA) is an autosome recessive genetic neuromuscular disorder, which is caused by deletion or mutation of *SMN1* (survival motor neuron1) gene. *SMN* exon 7 splicing is regulated by a number of exonic and intronic splicing regulatory sequences and the trans-factors that bind them. Here we identified a natural variant c.863G>T (p.Arg288Met) in exon 7 of SMN1 gene in three patients affected with type Ior typeII SMA .The in vivo assay showed that most of the *SMN1* transcripts loss the entire exon 7. The ex vivo splicing assay had demonstrated that this variant disrupt inclusion of exon 7 (about 85%) into *SMN1* mRNA and different bases replacement showed different splicing effect in both *SMN1* and *SMN2* pre-mRNA. The variant c.863G>T is located in the region that inclu-

des multiple splicing factors and is specially adjacent to the binding site of Tra2β1. Our results showed that this variant disrupts the binding of Tra2β1 not promote the binding of hnRNP A1. This present research about this natural occurrence variation influence the pre-mRNA splicing of SMN should be contributed to interpretation of the pathogenic mechanism in SMA patients.

Funding

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PS10.35

STIM1 mutations at a common amino acid residue (p.340) identified in two individuals with a predominant muscle disease phenotype

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Background: Dominant mutations in STIM1 have been identified in the complex phenotype Stormorken Syndrome (SS) and in non-syndromic tubular aggregate myopathy (TAM). All reported individuals with SS have a common p.R304W gain of function mutation in the coiled coil domain 1 of STIM1. In contrast mutations in TAM are restricted to the EF hand domain.

Methods and Patients: We performed exome sequencing on patient 1 and identified a de novo STIM1 mutation. We subsequently selected 4 patients with tubular aggregates, and 4 patients selected by phenotype, and performed immunostaining for STIM1 and direct sequencing of the STIM1 gene. Patients with STIM1 mutations were deep phenotyped.

Results: Two patients with STIM1 mutations were identified. Patient 1 has the common SS mutation (p.R304W), and exhibits features in keeping with this (thrombocytopaenia, miosis, aspenia, hypocalcaemia). Tubular aggregates were present in muscle biopsy and showed accumulation of STIM1. Patient 2 has a novel mutation at the same amino acid residue (p.R304G), and presents with a strikingly similar pattern of neuromuscular phenotype but aside from miosis no additional features of SS. The neuromuscular phenotype in both patients comprises myalgia, muscle stiffness, and reduction in range of joint movement, with mild weakness on examination.

Conclusion: The use of STIM1 immunoananlysis in patients with tubular aggregates was successfully applied to screen for patients with STIM1 mutations. We report a novel mutation at the common SS amino acid residue in a patient with TAM and miosis, in addition to further characterisation of a new patient with SS.

PM10.36

Clinical targeted exome (gene panel) approach advances the diagnosis of myopathies.

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We developed a massively parallel sequencing approach in order to optimize clinical diagnosis of myopathies at La Timone Hospital (Marseille), which is a reference center for a number of neuromuscular disorders in France. In a pilot project, we selected 298 genes implicated in neuromuscular diseases and cardiomyopathies listed in the Gene Table of Neuromuscular Disorders as well as differential diagnosis genes. Targeted exome approach was developed using HaloPlex target enrichment system adapted for Ion Torrent Next Generation Sequencing technology. This initial study had 92% sequence coverage at 20x and resulted in 47% diagnostic yield in a cohort of 37 distal myopathy patients. Detailed analysis of the technical characteristics of the study allowed us to pinpoint the aspects that could be further optimized. For example, we observed that longer HaloPlex probes were lost at the emulsion PCR step. Moreover, results for several target genes were not interpretable due to high rate of off-target read alignment. Based on these results, an optimized set of HaloPlex probes for 306 genes was designed for Illumina sequencing technology. Forty six myopathy patients were screened for mutations using this improved targeted exome approach. We believe that the diagnostic pipeline described in this study could be useful for other groups studying neuromuscular diseases. Moreover, the targeted exome approach used here could be applied to other genetic disorders.

PS10.37

Transthyretin (TTR) mutations in the turkish familial amyloid polyneuropathy (FAP) patients.

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- Introduction

Familial amyloid polyneuropathy (FAP) is an clinically heterogeneous autosomal dominant trait. Mutations in the TTR gene are most commonly observed. TTR is synthesized mainly in the liver and mutations in the gene result in misfolding and depositions of mutated TTR protein, cousing a life-threatening nerve length-dependent polyneuropathy. Until now, over 100 mutations are described that are reflected in the protein structure.

- Materials and Methods

Blood for DNA extraction was drawn after the patients signed an informed consent. DNA was isolated from 10ml whole blood using a commercially aviable kit (QIAamp DNA Blood Maxi Kit- Qiagene). For the detection of variants in *TTR* gene, coding region of the TTR gene was amplified and for the PCR fragments Sanger sequencing was performed (Macrogene). The obtained chromatograms were analysed with the aid of Chromas and CLC Main Workbench 5.5 programs. The obtained sequences were alined with the human reference sequence NG_009490. Detected variants were searched and confirmed in public databases such as *dbSNP* (http://www.ncbi.nlm.nih.gov/SNP/) and *Mutations in Hereditary Amyloidosis* (http://amyloidosismutations.com/cdna-attr.html).

- Results

Six different variants in theTTR gene were observed (n=48) (rs1800458, rs28933979, rs121918090 , rs121918097, rs121918082 , rs36204272) in our patient population . Four of the variations were described as pathogenic mutations in the databases [n=3, p.Val50Met (rs28933979); n=2, p.Gly67Glu (rs121918090); n=1, p.Gly73Glu (rs121918097); n=1, p.Glu109Gln (rs121918082).

- Conclusions

As a mere reflection the clinical and genetic heterogeneity of the disease, DNA testing helped
resolve the diagnosis of the 14.5% of the patients.

PM10.38

Targeted next-generation sequencing reveals novel TTN mutations causing recessive distal titinopathy

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Tibial muscular dystrophy (TMD) is the first described human titinopathy. It is caused by mutations in the last two exons, Mex5 and Mex6, of titin gene (*TTN*). The first reported TMD mutations were dominant and the Finnish founder mutation, an 11-bp insertion/deletion in Mex6, caused a severe early-onset limb-girdle muscular dystrophy 2J (LGMD2J) when homozygous. Later we reported that not all TMD mutations cause LGMD when homozygous, but some rather cause a more severe TMD. We have now performed a targeted next-generation sequencing assay of myopathy related genes on six families with recessive distal titinopathy. Two novel TMD mutations in *TTN* were identified, which both seem to be recessive. Three of the families, Italian, Bosnian and Iranian, had a novel nonsense TMD mutation in *TTN* Mex5 in homozygous state or in combination with another nonsense mutation on the other allele. One Hungarian family had a novel missense TMD mutation in *TTN* Mex6 in combination with a frameshift mutation on the other allele. In addition one Spanish family had a previously reported Iberian TMD mutation in combination with a frameshift mutation and another Spanish family had a novel missense mutation in *TTN* A-band in combination with a nonsense mutation and a novel titinopathy phenotype. Family members with only one *TTN* mutation were healthy. According to our previous results the frameshift and nonsense mutations located upstream of the last exons of *TTN* cause degradation of mRNA through nonsense-mediated decay and mainly the other mutated *TTN* allele is expressed. This study was supported by a grant from the Jane and Aatos Erkko Foundation.

PS10.39

Type 0 Spinal Muscular Atrophy: further delineation of pre- and post-natal features in 16 French patients

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Objective: Spinal muscular atrophy (SMA) is caused by homozygous inactivation of the SMN1 gene. The SMN2 copy number modulates the severity of SMA. The 0SMN1/1SMN2 genotype, the most severe genotype compatible with life, is expected to be associated with the most severe form of the disease, called type 0 SMA, characterized by severe neurologic impairment from birth. The aim of the study was to review clinical features and prenatal manifestations in this rare SMA subtype.

Methods: SMA patients with the 0SMN1/1SMN2 genotype were retrospectively collected using the UMD-SMN1 France database. Data from 16 patients were reviewed.

Results: These 16 patients displayed type 0 SMA. Most patients, at birth, had profound hypotonia, severe muscle weakness, severe respiratory distress, and cranial nerves involvement (inability to suck/swallow, facial muscles weakness). They also showed characteristics of fetal akinesia deformation sequence (FADS) and congenital heart defects (CHD). Recurrent episodes of bradycardia were observed. Death occurred within the first month. At prenatal stage, decreased fetal movements were frequently reported, mostly only by mothers, in late stages of pregnancy; increased nuchal translucency was reported in about half of the cases; CHD, abnormal amniotic fluid volume, or joint contractures were occasionally reported.

Interpretation: Despite a prenatal onset attested by severity at birth and signs of FADS, prenatal manifestations of type 0 SMA are not specific and not constant. Finally, as illustrated by the frequent association with CHD, type 0 SMA physiopathology is not restricted to motor neuron, highlighting that SMN function is critical for organogenesis during development.

PM10.40

A novel single nucleotide splice site mutation in FHL1 confirms an Emery-Dreifuss plus phenotype with pulmonary artery hypoplasia and facial dysmorphology

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We describe a Danish family with an, until recently, unknown X-linked disease with muscular dystrophy (MD), facial dysmorphology and pulmonary artery hypoplasia. One patient died suddenly before age 20 and another was resurrected from cardiac arrest at the age of 28. Linkage analysis pointed to a region of 25 Mb from 123.6 Mb to 148.4 Mb on chromosome X containing over 100 genes. Exome sequencing identified a single nucleotide splice site mutation c.502-2A>T, which is located 5' to exon 6 in the gene encoding four and a half LIM domain 1 (FHL1) protein. FHL1 expresses three main splice variants, known as FHL1A, FHL1B and FHL1C. In healthy individuals, FHL1A is the predominant splice variant and is mainly found in skeletal and cardiac muscle. The FHL1 transcript profiles from two affected individuals were investigated in skin fibroblasts with quantitative real-time PCR. This demonstrated loss of isoform A and B, and an almost 200-fold overexpression of isoform C confirming that lack of FHL1A and overexpression of FLH1C results in an extended phenotype of EDMD as recently shown by Tiffin et al. (2013)

PS11.001

Chromosome 18p deletion syndrome: clinical, developmental and cytogenomic characterization of six Brazilian patients

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Introduction: Chromosome 18p deletion syndrome [del(18p)] (OMIM 146390) has been well described in literature with over 150 patients reported on, but few of them were evaluated by cytomolecular techniques.

Materials and Methods: We studied six Brazilian patients with 18p deletion based on clinical, developmental and cytogenomic findings. The patients were evaluated by a specific clinical protocol, including immunological, endocrinological and neuropsychological assessments. The cytogenetic study was performed by G-banding karyotype, SNP-array (Genome-Wide Human SNP Array 6.0, Affymetrix) and FISH-BAC techniques.

Results: The results showed four *de novo* pure deletions and two deletions caused by derivative chromosomes, one of them *de novo* and the other inherited from a paternal translocation. Although literature indicates 18p11.11 as the most frequent breakpoint, our patients presented different breakpoints: 18p11.21 (4/6, four out of six) and 18p11.23 (2/6). The main clinical findings were: short stature (5/6); microcephaly (2/6); ectopic pituitary (1/6); growth hormone deficiency (1/6); hypothyroidism (3/6); intellectual disability (5/6); cardiac anomalies (2/6); scoliosis (3/5); and *keratosis pilaris* (4/6). The distinguished facial dysmorphic features were similar among the patients: ocular hypertelorism (6/6); ptosis (3/6) and strabismus (6/6). The neuropsychological assessments showed IQ scores from borderline intellectual functioning to moderate intellectual disability.

Conclusions: Some genes located in the 18p deleted segment seem to play an important role in the patient's phenotype, such as *TGIF1, GNAL, LAMA1* and *LPIN2* genes. The SNP-array technique permitted a better chromosome breakpoint definition and, associated to the specific clinical protocol, provided a better genotype-phenotype correlation revealing genes that might influence the patient's phenotype.

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PM11.002

Three new cases with atypical deletions in the 22q11.2 region

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22q11.2 deletion syndrome (22q11.2DS) is mainly characterized by conotruncal congenital heart defects, velopharyngeal insufficiency, hypocalcemia and a characteristic craniofacial appearance. The etiology in the majority of patients is a 3-Mb recurrent deletion in 22q11.2 region. However, rare cases with proximal, central and distal deletions in 22q11 region have been reported with different phenotypes.

We report three cases with atypical deletions diagnosed in a total of 119 patients with deletions in 22q11.2 region attended in our 22q11DS Multidisciplinary Clinics.

The first case, a girl with short stature, hirsutism and ventricular septal defect was found to have a distal 22q11DS deletion, arr[hg19] 22q11.21(20754422-21561514)x1, including the CRKL gene and with no overlap with the known critical region. The deletion was also present in her brother that only presents nasal voice and her asymptomatic father. A paternal uncle died around 3 months old of congenital heart disease sixty years ago.

The second and third cases are two boys that shared a de novo deletion in the centromeric end of the distal region of 22q11DS, arr[hg19] 22q11.21q11.22 (21505358/21808950-22905068)x1. One patient had global developmental delay and an Autism spectrum disorder (ASD) while the other patient had global developmental and learning delay, with particular phenotypic features.

Correlation of clinical and genetic data of atypical 22q11DS deletions is of

cardinal importance in dissecting the contribution of individual genes to DGS phenotype. In particular, nasal voice cosegregation with a partial deletion of 22q11DS region (case 1) may point to specific genes involved in palate formation or function.

PS11.003

Delineation of a novel 2q11.1q11.2 Microduplication syndrome

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Using chromosomal microarray testing we detected a 1.48 Mb *de novo* 2q11.1q11.2 duplication in a patient with developmental delay, microcephaly, plagiocephalus, torticollis, laryngomalacia and failure to thrive. This duplication contains about 26 genes (12 OMIM genes). One *de novo* duplication 2q11.2 with a minimum size of 1.47 Mb was described in the literature in a patient with moderate developmental delay, hypotonia with continued head lag at age 15 months, short stature, midface hypoplasia with hypoplastic nose, macrocephaly with frontal bossing and dolichocephaly and significant shortening of all segments of the extremities with significant brachydactyly. Five other patients with similar duplications and psychomotor retardation/ learning difficulties were found through the DECIPHER database. While head circumference seems to be variable, shared anomalies consist of frontal bossing/ large forehead (described in 4 of 7 patients), birth weight < P10 and failure to thrive (described in 2 patients), muscular hypotonia (described in 2 patients), sensorineural hearing impairment (described in 2 patients) and café-au-lait spots (described in 2 patients). In 3 cases the duplication was maternally inherited (in 2 cases from mothers with known learning difficulties), in 3 cases the duplication was *de novo* and in one case the inheritance was unknown because the father could not be tested. In one patient another *de novo* aberration was detected, which could have an impact on the phenotype. Our observations indicate that 2q11.1q11.2 duplication is a novel recurrent microduplication syndrome mediated by flanking low-copy repeats.

PM11.004

A 17 mb deletion in 2q31.3-q33.1 in a girl with central nervous system malformations and severe developmental delay

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More than 30 cases with a deletion of the 2q31-q33 region have been reported. Deletions vary in size and location and are associated with variable clinical features including craniofacial dysmorphism, developmental delay, growth retardation and other anomalies. We report the case of a girl born from healthy unrelated parents after an uncomplicated pregnancy (birth weight 2660g). Perinatal history was unremarkable, apart from a congenital laryngeal stridor. Her motor development was delayed (sit position at the age of 3 years, walk at the age of 4 years). Dysmorphic features were evidenced: triangular face, high forehead, maxillary hypoplasia, small eyes, low set ears, short neck, small teeth, small hands. Ophthalmological examination showed alternating strabismus. The girl developed epilepsy from 6 month of age, resistant to drug treatment. Neuroradiological examination at the age of 9 and 10 years showed a diffused reduction of white matter with moderate ventriculomegaly, gracile posterior body and splenium of corpus callosum and gracile hippocampus. Neurological examination evidenced based enlarged deambulation, chaotic movement and dysmetria, no other anomalies. She developed motor stereotypies of the hands, severe intellectual disability (not able to speak), and aggressive behavior both self- and hetero-aggressive. In past clinical history reported frequent respiratory, skin and oral infection (no specific immunological deficit). Array CGH analysis has evidenced a 17 Mb deletion in chromosome in 2q31.3-q33.1. Our case shows phenotypic similarities with previously described cases with overlapping deletions; in addition we report the presence of a drug resistant epilepsy, reported also by Mencarelli (2007) and Mitter (2010), and of previously unreported brain anomalies, behavior problem and recurrent infections.

PS11.005

2q33.1q34 microdeletion syndrome: a case report

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We describe a case of a girl, the second child of healthy non-consanguineous parents. Pregnancy was complicated by threats of preterm birth, but she was born full term. Birth weight was 3,120 kg and length 50 cm. At birth she had anterior anus with rectum perineal fistula, left hip dysplasia and several dysmorphic features: bilateral blepharophimosis, up-slanting palpebral fissures, epicanthus inversus, low-set ears, micrognathia, mild macroglossia, long hands and fingers (+2DS). She presented feeding difficulties, failure to threat and mild psychomotor delay. Brain MRI performed at 2 months of age was normal. She had also bicuspid aortic valve and selective IgA deficiency with recurrent respiratory and gastrointestinal infection. Array-CGH showed a *de novo* microdeletion of 8,3 Mbp in 2q33.1q34 (201,315,087-209,635,377). This region contains some interesting genes, such as CD28, CTLA4 and ICOS, that are important regulators of the immune system.
This microdeletion can explain the clinical features of our patient, outlining the characteristics of this syndrome.

PM11.006

Cerebral white matter abnormalities in 6p25 deletion syndrome

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We report the phenotype and genotype of a 4 years and 11 months old boy who was transferred to hospital after an accident. CT-scan and cMRI showed a cranial fracture as a result of the accident and two incidental findings: 1) large bifronto-temporal arachnoidal cysts and 2) multifocal white-matter-lesions involving the periventricular, deep, and subcortical cerebral white matter.
The clinical phenotype of the boy included mild developmental delay, strabism, slightly dysmorphic features and mild hearing impairment.
Molecular karyotype from peripheral blood detected a ~ 3,9 Mb heterozygous microdeletion in 6p25.3 - 6p25.2 deleting 34 genes, 18 of which were OMIM annotated including the transcription factor FOXC1.
Microdeletions in 6p25 are thought to be associated with a distinctive clinical phenotype - the 6p subtelomeric deletion syndrome - that includes sensorineural hearing loss, anterior chamber eye defects, cardiac defects, developmental delay and other developmental and behavioral abnormalities, hypotonia, hip dysplasia, cerebellar abnormalities and a characteristic facial appearance. Few reports exist that describe multifocal T2-weighted and FLAIR abnormalities involving the periventricular, deep, and subcortical cerebral white matter associated with the 6p25 deletion in young children and in adults.
From our case and from the review of the literature we conclude that a microdeletion in 6p25 should be considered as the cause for unclear multifocal cerebral white matter abnormalities in patients that in addition have dysmorphic features and multiple congenital anomalies even when intellectual abilities are within the normal range (Vernon et al. 2013).

PS11.007

9q34.3 microdeletion in three patients characterized by array-CGH

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Submicroscopic deletion del(9)(q34.3) is a newly described genomic disorder that has been reported as a clinically recognizable 9q Subtelomeric Deletion Syndrome (9qSTDS) also known as 9q34.3 Microdeletion Syndrome and Kleefstra Syndrome. That microdeletion affects fetal development and results in intellectual disability, childhood hypotonia and facial feature. In addition, congenital heart and renal defects, microcephaly, epilepsy, obesity, behavioral problems and absence of expressive speech are frequently present. Structural brain and/or subcortical white matter abnormalities may also occur. Microdeletions of the 9q34.3 region, like other terminal deletions, have breakpoints occurring in multiple sites of the distal chromosome end. There is no evidence for phenotype-genotype correlation between size of the deletions or type of mutations and severity of clinical features. Studies confirm the *EHMT1* gene to be the major determinant of the 9qSTDS phenotype. In addition, *EHMT1* haploinsufficiency is associated with neurodegeneration and neurodevelopmental defect. We report the molecular

characterization and phenotype of three patients, 3-years-7-months-old, 1-year-7-months-old, and 2-years-old, with respectively 374 Kb, 867 Kb and 1,750 Kb terminal deletions at 9p34.3 chromosome region detected by array-CGH. Although they had different deletion size and OMIM Genes deleted, they present similar and recognizable phenotype of 9q34.3 microdeletion syndrome. The first patient has only *EHMT1* and *CACNA1B* genes deleted, but all they have a submicroscopic deletion that share an overlapping region encompassing those genes. It reinforces that haploinsufficiency of both genes *CACNA1B* and *EHMT1* are dosage-sensitive and contribute to core phenotype of 9qSTDS.

PM11.008

Microdeletion of 9q34.11: an emerging contiguous gene syndrome?

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Interstitial microdeletions within 9q34.11 have been described in a relatively small number of patients to date. Recurrent clinical features reported in these individuals include intellectual disability, hypotonia and seizures amongst others. Deletions including the STXBP1 gene have been associated with an increased risk of epilepsy. However, further genotype-phenotype correlations remain poorly defined. Here we describe two previously unreported patients with interstitial deletions of this region. In patient 1, a de novo deletion of ~1.8 Mb was detected at 9q33.3q34.11. Although this deletion is proximal to the Kleefstra syndrome region at 9q34.3, it appears to result in a strikingly similar phenotype including progressive microcephaly, hypotonia, severe developmental delay and facial dysmorphism. In patient 2, a deletion of ~2.1 Mb of 9q34.11 was detected. Clinical features noted in this patient overlap those described in patient 1 and include speech and language delay, microcephaly, hypotonia and facial dysmorphism. Importantly, absence seizures were reported in patient 2, despite the STXBP1 gene not being deleted in this individual. A common region of ~885 kb containing approximately 28 genes was deleted in both patients. These case reports provide further evidence for an emerging 9q34.11 contiguous gene deletion syndrome characterised by developmental delay, microcephaly and hypotonia and may assist in refining genotype-phenotype correlations associated with deletions of this region.

PS11.009

Mutation analysis in patients with Adams-Oliver syndrome using a targeted resequencing strategy.

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Adams-Oliver syndrome (AOS) is a rare developmental disorder typically characterized by congenital cutis aplasia of the scalp and vertex with transverse terminal limb defects. In addition, vascular anomalies, comprising pulmonary and portal hypertension, retinal hypovascularisation and congenital cutis marmorata telangiectasia, as well as congenital heart defects, such as ventricular septal defects, tetralogy of Fallot and valve anomalies have been observed.

To date, five causal genes have been described: EOGT and DOCK6 for recessive AOS and ARHGAP31, RBPJ and NOTCH1 in dominant AOS and sporadic cases.

Using a targeted resequencing strategy with HaloPlex Targeted Enrichment (Agilent) and sequencing by synthesis on MiSeq (Illumina), the five known AOS genes were analyzed in 66 patients and families with an AOS phenotype or isolated aplasia cutis.

Possible causative variants were identified in 17 families. The majority of mutation positive families harboured a NOTCH1 mutation (7 families), followed by DOCK6 (5 families), EOGT (3 families) and RBPJ (2 families). No ARHGAP31 mutations were deteceted.

In conclusion, a mutation in circa 25% of the aplasia cutis/AOS families was detected with a next-generation based targeted resequencing approach of five known AOS causing genes. This confirms the genetic heterogeneity of AOS/aplasia cutis, and highlights a large proportion of patients with still unexplained etiology.

PM11.010

Webb-Dattani Syndrome (WEDAS), Report of a Saudi Arab Family with a Novel Homozygous Mutation in the *ARNT2* Gene

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Webb-Dattani syndrome (#615926) is an autosomal recessive disorder characterized by frontotemporal hypoplasia, pituitary and hypothalamic insufficiency due to hypoplastic development of these brain regions. The condition was described in 6 siblings from highly consanguineous Saudi Arabian kindred with congenital brain malformation, panhypopitutrism, acquired microcephaly, seizures, severe global developmental delay, spastic cerebral palsy, and post-retinal blindness. A homozygous truncating mutation was identified in the *ARNT2* gene by a combination of homozygosity mapping and whole exome sequencing.

Here we describe another family with three affected siblings born to a healthy Saudi Arab first cousins couple. The index patients were identical male twins presented at three months of age with congenital central hypotonia and hypoventilation precipitated by an acute bronchiolitis. They had a similar clinical manifestation that was characterized by diabetes insipidus, central hypothyroidism, adrenocortical insufficiency, severe developmental delay, acquired microcephaly, cortical blindness with normal retinal examination, seizures, and gastroesophageal reflux. Brain MRI showed hypothalamic, infundibular, and pituitary axis insufficiency, small sella with absent posterior pituitary bright spot. Family history is significant for an older affected deceased female sibling at 18 months of age, and four miscarriages of undetermined etiology. Whole exome sequencing detected a homozygous unclear variant (c.378C>Tp.G126G) in ARNT2 gene of both of the affected twins. According to splice prediction programs, this variant creates a new donor splice site, possibly leading to a loss of function. Both of the parents were found to be heterozygous and none of the unaffected siblings were homozygous for this variant.

PS11.011

Delineating a new interstitial genomic rearrangement by array-CGH at 19p13.3 band

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Introduction: High resolution microarray comparative genomic hybridization (aCGH) is a powerful genetic tool implemented as a first-tier test for diagnosis of genomic imbalances in individuals with intellectual disability, autistic disorders and congenital malformations.it also has been proven to be successful in characterizing the growing list of microdeletion/duplication syndromes. Using this experimental approach, we and others recently described new microdeletion/microduplication syndromes.

Methods: A "genotype first" approach, using aCGH, in which patients are characterized by a similar genomic rearrangement before a common clinical presentation has been used.

Results: We report 13 new patients with proximal 19p13.3 submicroscopic rearrangements and review patients from the literature and public genomic databases such as DECIPHER and ISCA Consortium for a total of 37 cases. We describe the phenotypic findings and suggest these patients represent a new microdeletion/duplication syndrome at 19p13.3 band, with a 113.5 Kb critical region as Small Region of Overlapping harboring three genes. Common features consist of abnormal head circumference in most patients (macrocephaly with the deletions and microcephaly with the duplications), intellectual disability (ID) with developmental delay, hypotonia, speech delay and common dysmorphic features.

Discussion: This study provides detailed clinical information for geneticists to assist in the evaluation, diagnosis and management of individuals with similar genomic interstitial rearrangements at 19p13.3 band.

PM11.012

Genetic diagnosis of chromosomal anomalies in pediatric patients by array CGH.

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Laboratory evaluation of patients with developmental delay/intellectual disability, congenital anomalies and dismorphic features has changed significantly in the last years with the introduction of microarray technologies. With these techniques, a patient`s genome is examined for detection of gains or loses of genetic material that typically are too small to be detectable by standard G-banded chromosome studies.

In our laboratory this technology has been implanted as the first tier test for genetics studies for developmental delay/ mental retardation/ autistic disorder and/or multiple congenital anomalies (DD/MR/ASD/MCA).
In this study we present the results of array CGH obtained in 548 patients with clinical diagnosis of DD/MR/ASD/MCA. The Nimblegen CGX Cytogenetic Microarrays platform, suplied by PerkinElmer, was performed.
From the total of 548 patients, the 57,1% were men and 42,9% women. Patologic result was obtained in 13,1% of patients, without stadistical diferences between sexs.The genetics diagnostic yield obtained were 12,5 % for developmental delay, 10,1% for mental retardation, 10,7 for autistic disorder and 16,8 for multiple congenital anomalies.
Previous studies suggest that when array-CGH is performed, the diagnostic yield reached 8 to 20 % of total patients. Our results are in concordance wiht these previous results with an average of 13,1% detected from the total of patients, with a range from 10,1% in the case of mental delay to 16,8 for multiple congenital anomalies.
Our results are in concordance with the results previously obtained in other laboratories and they are improved significantly the genetics diagnosis for DD/MR/ASD/MCA patientes in our laboratory.

PS11.013

Progressive cerebral striatal arteriopathy in ichthyosis prematurity syndrome; is it eosinophilia-related?

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BACKGROUND Ichthyosis Prematurity Syndrome (IPS) is characterized by premature birth, thick caseous desquamating epidermis, respiratory complications and transient eosinophilia. Although respiratory problems can be severe, outcome after the first months is reported to be favorable. Mutations in FATP4 have been identified in this rare autosomal recessive disorder.
METHODS We report on non-identical twin brothers with consanguineous parents born prematurely at 30+4 gestational weeks, both with congenital skin lesions affecting the thorax, back and extremities, but most prominently the scalp. After birth, CPAP was needed for two days due to respiratory distress. At 6 weeks of age, intubation was necessary for 4 days in one boy due to apnea and desaturation. Serial brain ultrasound in both boys showed progressive striatal arteriopathy. Blood eosinophilia was demonstrated in both patients. At 2 months, the skin lesions improved tremendously, leaving only a reddish discoloration and dryness of the skin.
RESULTS Initial skin pathology showed a verrucous epidermis and hyperkeratosis with corneal desquamation. Targeted genetic analysis revealed the presence of the homozygous c.469A>G change leading to the p.Asn157Asp amino acid substitution in the FATP4 gene, confirming the diagnosis of IPS in both boys.
DISCUSSION Progressive striatal arteriopathy has not been described in IPS. Both boys showed eosinophilia, which can induce brain damage. In addition, encephalopathy is a known feature in eosinophilia-associated conditions, i.e. Idiopathic Hypereosinophilia syndrome (OMIM 607685), Incontinentia Pigmenti (OMIM 308300) and autosomal recessive Hyper-IgE syndrome (OMIM 243700). We hypothesize that hypereosinophilia may have been a causative factor for the striatal arteriopathy, which may be an underrecognized finding in IPS.

PM11.014

A 60Kb deletion in the AUTS2 gene in a patient with cardiopathy, facial dysmorphism and autistic traits

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We present the case of a child referred to the Dismorphology consultation at the age of 3 months. Born after normal pregnancy and instrumental delivery at 36+5 w. Birth weight: 2690g and Apgar 7/9. At 4 hours of life he presented an episode of choking while sucking that needs stimulation for recovery. Progressively acquires suck-swallow coordination and no significant symptoms associated were observed. An atrial septal defect (ASD) (ostium secundum) was diagnosed by echocardiography and the patient was discharged for control in external consultations.
On the exploration at 3m slightly decreased axial, lower limbs on flexion (without arthrogryposis), increased tendon reflexes, and peculiar phenotypic traits (hypertelorism and almond shaped palpebral fissures, short philtrum, thin upper lip and microstomia) were observed.
At 15 months, the ASD was repared. He presented a convergent not paretic strabismus, the muscle tone was improved in general but the lower limb tone was increased when excited, the reflexes were symmetrical but exalted and the CPR was extensor bilateral. We observed a limitation of joints extension which improved in subsequent controls. Now, at 41momths, he presented a global developmental delay with autistic traits.
The aCGH (Nimblegen, 720K) performed defined a 60Kb deletion on 7q11.22, not seen in the aCGH (qChip [R], 60K), and confirmed by MLPA in the AUTS2 gene.
Balanced genomic rearrangements disrupting AUTS2 gene have been identified implicating AUTS2 in neurodevelopmental disorders. More recent descriptions suggest that the AUTS2 syndrome could be a single gene disorder and our case support this hypothesis.

PS11.015

Familial translocation t(1;12)(q43;q21.1) truncates a CHRM3 GENCODE isoform in a family with specific learning difficulties, ataxic symptoms, and stroke

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We have established a National Registry of Balanced Chromosomal Rearrangements in Finland by ascertaining all known Finnish carriers (n=2575). The registry contains medical records of all available carriers including family members carrying the same rearrangement identical-by-descent. From this resource, we have drawn families with apparent correlating phenotype to the balanced translocation. In our gene-mapping pilot we identified a potential positional candidate gene for intracranial and aortic aneurysm (Luukkonen et al. 2013, JMG).
Here, we describe the clinical and genetic findings in a family where carriers (n=6) of t(1;12)(q43;q21.1) are affected first by specific learning difficulties, chronic headache, balance problems, tremor, fatigue, and later with stroke without known predisposing factors. By next-generation mate-pair sequencing, we fine-mapped the specific chromosomal breakpoints, which do not truncate any protein coding genes. On chromosome 1, *CHRM3* locates 62 kb distal to the breakpoint, and a *CHRM3* GENCODE isoform spans the breakpoint. Significant linkage for episodic ataxia type 3 has been previously identified in 1q42.3. On chromosome 12, *ATXN7L3B* locates 550 kb and *KCNC2* 1.2 Mb distal to the breakpoint. Both genes were within a 670 kb deletion in a recently published family with a complex neurodevelopmental and ataxic phenotype.
This study has brought into focus three genes - *CHRM3*, *ATXN7L3B* and *KCNC2* in a family with an early retirement because of complex neurological phenotype leading to stroke, and perfectly co-segregating translocation t(1;12)(q43;q21.1). We propose that disruption of the *CHRM3* GENCODE isoform may play a role in the pathogenesis and lead to the observed complex phenotype.

PM11.016

Is Bardet-Biedl syndrome more frequent in Europeans than current estimates?

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It is generally quite difficult to have reliable estimates of the prevalence of rare genetic diseases as they may be often underdiagnosed and there are few efforts to have systematic data collection on a scale large enough to obtain significant numbers. Often numerical values are quoted either from rare ancient studies or even unreferenced. In the case of Bardet-Biedl syndrome, a genetically heterogeneous disease, the numbers usually quoted are 1:125 000 to 1:160 000 for European populations. While Bardet- Biedl syndrome can be caused by mutations in 19 currently identified genes (OMIM February 2015), two genes (BBS1 and BBS10) account for almost half the cases in European populations, and each of them have a major mutation, p.Met390Arg for BBS1 and p.Cys91LeufsTer5 in BBS10. We have looked for the frequency of these two mutations in the ExAC database, and for the cu-

mulated frequency of other rarer known mutations in these genes. Based on this analysis, we estimate that the frequency of BBS in Europeans is likely to be about 1:50 000, ie 3 times more than previous estimates, and this suggests that this severe and pleiotropic disease was underdiagnosed.
Using the same strategy of using a recurrent mutation whose contribution to a genetically heterogeneous disease has been well established, we estimated the incidence of Aicardi-Goutières syndrome (that was quoted as unknown in the most recent update of the cognate GeneReviews) at about 1:50 000 to 1:65 000, using ExAC data for the p.Ala177Thr mutation in the RNASEH2B gene.

PS11.017

Identification of a new mutation confirms the implication of IFT172 in Bardet-Biedl Syndrome (BBS20)

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Introduction: Bardet-Biedl Syndrome (BBS; MIM 209900) is a recessive and genetically heterogeneous ciliopathy characterized by postaxial polydactyly, retinitis pigmentosa, obesity, hypogonadism, cognitive impairment and kidney dysfunction. So far, 20 BBS genes have been identified, with the last ones reported being found in one or few families.
Materials and Methods: Exome sequencing was performed in a consanguineous family in which two affected children presented typical BBS features (retinitis pigmentosa, postaxial polydactyly, obesity, hypogonadism and cognitive impairment). *BBS1* to *BBS18* were sequenced by New Generation Sequencing (*BBS1* to *BBS16*) and Sanger Sequencing (*BBS17* and *BBS18*).
Results: A missense mutation in *IFT172* gene (NM_015662.2: c. 2857C>T, p. Arg953Cys) was identified at the homozygous state in the two patients and found at the heterozygote state in both parents and their 3 healthy children. *IFT172* mutations have been initially reported in Jeune and Mainzer-Saldino syndromes (Halbritter et al., 2014). Recently, mutations have also been found in isolated retinitis pigmentosa and Bardet-Biedl- like ciliopathy (Bujakowska and al., 2014). This is the second report of *IFT172* mutations in BBS patients confirming *IFT172* as a BBS gene. Moreover, another *IFT* gene, *IFT127*, was already associated with Bardet-Biedl syndrome (Aldahmesh et al., 2014) and this report confirmed the implication of IFT genes in the pathogenesis of BBS.
Conclusions: In this report we validate *IFT172* as the 20th BBS gene (BBS20) and confirm intraflagellar transport defects in BBS.

PM11.018

Characterization of mutations in BBS5 gene by functional analysis in zebrafish model

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Introduction: Bardet-Biedl syndrome (BBS, #209900) is a rare genetic disorder considered a member of the group of ciliopathies. In recent years, zebrafish (*Danio rerio*) has been extensively used as a genetic model for the study of human disorders given the high homology that exists between human and zebrafish genomes (>80%). Here, we report an *in vivo* assay to analyse the functionality of three *BBS5* variants, predicted to be pathogenic, which have been detected by WES or direct sequencing.
Materials and Methods: Functional modelling of *BBS5* variants was carried out using 8-10 somite stage embryos, previously microinjected with bbs5-MO at one- to two-cell stage. Non-injected embryos were used as controls. We therefore performed rescue experiments with full-length human mRNA (wild-type and mutant, separately). Thus, affected embryos were classified according to severity of observed phenotypes.
Results: As previously reported, zebrafish embryos injected with bbs5-MO manifested several gastrulation defects, such as curved body axis, kinked notochord, longer somites or partial loss of somite definition. These phenotypes, ranging from mild to more severe, seem to be rescued by using wild-type human BBS5 mRNA, but not with mRNA carrying the detected mutations.
Conclusions: Our preliminary results indicate that these mutations could cause defects that lead to an abnormal planar cell polarity (PCP) signalling, which is known to underlie certain clinical phenotypes in BBS patients. These findings need to be confirmed by whole mount in situ hybridization (ongoing).
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PS11.019

Characterization of the total ciliopathy variant load dissolves the enigma of oligogenic inheritance in Bardet-Biedl syndrome

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Bardet-Biedl syndrome (BBS) is clinically and genetically heterogeneous and overlaps with other ciliopathies. Mutations in about 20 genes are described. BBS was among the first Mendelian disorders for which triallelic inheritance has been proposed which still causes uncertainty for genetic counselling and prenatal diagnostics. We performed genetic testing in 145 unrelated BBS patients, the most comprehensive sequencing-based study to date. Initially, we tested for the *BBS1* and *BBS10* hotspot mutations. NGS using our panel for ciliopathies (currently targeting 381 genes) was performed in 81 patients. In all but three families who fulfilled the diagnostic criteria, we identified homozygous or compound heterozygous mutations in a single *BBS* gene or *ALMS1*. Most mutations were private, 50 were novel and not described so far. High-coverage NGS enabled the detection of causative CNVs which were key to the diagnosis in hitherto unsolved constellations. As no MLPA kit is available for any of the *BBS* genes, all deletions would have been most probably missed by conventional techniques. Most patients carried additional mutations at other loci. However, in contrast to published data, our findings are in accordance with a recessive disease model. While modifiers may play a role for variable expressivity, our study widely resolves the long-standing enigma of triallelic or oligogenic inheritance in BBS. More than 95% of typical BBS patients harbour pathogenic mutations in one of the known disease genes why we conclude that further genetic heterogeneity is limited. Our data is of major importance for genetic counselling, clinical management and prenatal diagnostic testing.

PM11.020

Beals-Hecht syndrome: expanding the clinical phenotype

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Introduction: Beals-Hecht syndrome (BHS) or Congenital ContracturalArachnodactyly (CCA) is a rare autosomal dominant connective tissue disorder characterized by dolichostenomelia, crumpled ears, arachnodactyly, muscle hypoplasia andmultiple joint contractures. Heterozygous mutations in FBN2, which codes for fibrillin-2, are identified in about 27-75% of patients.In animal models,FBN2 is a key regulator of bone formation during embryogenesis.
Case presentation: We describe a 2 year-old boy, who is the second child of healthy non-consanguineous parents. At birth,micrognathia, crumpled helices, pectuscarinatum, arachnodactyly and campodactyly of both hands and feet, flexion contractures of wrists, elbows and knees, and generalized muscle weakness were noticed. Cardiac evaluation identified total anomalous pulmonary venous return and patent ductus arteriosus. Chest x-ray showed bilateral fusion of multiple ribs.
ArrayCGH did not identify pathogenic CNVs. Sequencing of FBN2 revealed two novel heterozygous variants: c.7138+1G>A,at a donor splice site, and a missense variant of unknown significance, c.2934T>A(p.Phe978Leu). Segregation analysis is ongoing.
Conclusion:Our case is within the most severe clinical spectrum of BHS/CCA.To our knowledge, apart from an infant with partial fusion of C2-C3 vertebral bodies, no other bone fusions were described in affected humans [1]. We propose the fused ribs present in our patientcould be an additional clinical finding in relation to BBS/CCA. Bioinformatic evaluation indicated that the c.7138+1G>A variant affects splicing of exon 56, and is thus probably pathogenic. Nonetheless, functional studies and results from segregation

analysis are needed to better clarify the pathogenicity of the FBN2variants identified in this patient.

PS11.021

Relation between assisted reproduction techniques and genomic imprinting defects

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Introduction: Beckwith-Wiedemann (BWS) is an overgrowth syndrome characterized by an unusual growth, macroglossia, hemihyperplasia, and other features as abdominal wall defects, major frequency of tumors compared with general population. Other clinical features are embryonic tumors, adrenocortical cytomegaly, ear anomalies, visceromegaly, renal abnormalities, neonatal hypoglycemia and polydactyly.
The molecular basis of this syndrome is multifactorial, but the most common alterations are imprinting defects at 11p15.5 locus, which represents about 70% of the cases.
Material and Methods: In this study, we analyzed KvDMR and H19DMR methylation levels in 121 patients with BWS, 16 born by assisted reproductive techniques (ART) and 105 by natural conception.
In 25 patients (15 from ART and 10 from Natural conception) we also analyzed several methylation imprinting loci: SRNPN, PEG1, ZAC, and GNAS. MS-MLPA and pyrosequencing were applied to performed molecular analysis of the imprinting regions.
Results: We found alterations in the locus 11p15.5 in 15 of 16 patients born by ART and 61 of 105 of patients born by natural conception. In addition, several patients born by assisted reproductive techniques presented defects in other imprinting loci.
Conclusions: Imprinting defects in patients born after assisted reproductive techniques are nine times more frequent than patients born after natural conception. This defects may include more than one imprinting loci and the dysregulation of the epigenetic pattern are related to the development of imprinting disorders such as Beckwick Wiedemann. Thus, ART is related to the disruption of DNA methylation pattern.
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PM11.022

Epigenetic and genetic defects in Polish patients with Beckwith-Wiedemann syndrome

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Beckwith-Wiedemann syndrome (BWS) is a disorder characterized by pre- and postnatal overgrowth in children, abdominal wall defects, macroglossia and a high risk of tumors. It is caused by different epigenetic and genetic defects of the 11p15 region containing imprinted genes which are localized in two domains controlled by the imprinting control region IC1 and IC2.
The aim of the study was to establish the molecular background of BWS in a group of 77 Polish patients. Molecular analyses were performed on leukocyte DNA and comprised methylation sensitive multiplex ligation-dependent probe amplification (MS-MLPA), microsatellite analyses, *CDKN1C* gene sequencing, and arrayCGH. The presence of 11p15 defects in 60 BWS patients, including a pair of monozygotic twins, was revealed. Hypomethylation at IC2 in 36 patients, hipermethylation at IC1 in 3 patients, and paternal UPD of 11p15 in 12 patients were identified. In other 7 patients aberrant methylation was associated with the presence of CNVs: duplications, a triplication or deletions involving both or one of the imprinted domains. In two patients novel frameshift mutations in *CDKN1C* gene were found. In the majority of cases genetic defects (CNVs, *CDKN1C* mutations) were inherited and their effects depended on their size, localization and the parental inheritance. Overall, the study revealed the presence of epigenetic or genetic defects in 77% of investigated patients. The pattern of our molecular findings is comparable with other populations. The study provides detailed data concerning mutational spectrum of a significant group of BWS patients.
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PS11.023

Brain-thyroid-lung syndrome in a child caused by a deletion on chromosome 14 proximal of the NKX2.1 gene locus

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Brain-lung-thyroid syndrome (BLTS) is a rare disorder characterized by congenital hypothyroidism (CH), infant respiratory distress syndrome (IRDS) and benign hereditary chorea (BHC). BLTS is caused by mutations in the *NKX2.1* gene, a transcription factor which is involved in the development of the thyroid, lung and central nervous system.
In previously documented and published cases of patients with brain-thyroid-lung syndrome a mutation or deletion of *NKX2.1* has always been present. Here we present one patient with the clinical features of brain-thyroid-lung syndrome without a mutation in *NKX2.1*. Using *array comparative genomic hybridization*, we identified a heterozygous 1 Mb deletion on chromosome 14, which is approximately 195,000 bp proximal to *NKX2.1*. The deleted region encompasses 3 genes: *MBIP*, *BRMS1L* and *RALGAPA1*. Currently, we are trying to determine whether NKX2.1 directly interacts with one of these 3 proteins or whether one of the three proteins directly binds to the *NKX2.1* gene promoter.
Recently, a heterozygous deletion 200 kb proximal to the *NKX2.1* gene has been identified in another patient with a choreiform movement disorder. The smallest common deleted region of our patient and the described patient encompasses just the *MBIP* gene (MUK-binding inhibitory protein). MBIP is expressed in the thyroid, lung and forebrain. Our current aim is to clarify whether MBIP is involved directly or indirectly in *NKX2.1* gene expression or regulation. This finding will help to identify completely new mechanisms of NKX2.1 regulation and provide a deeper understanding of the thyroid development and the possible cause of thyroid disorders.

PM11.024

Exome sequencing reveals Camptosynpolydactyly and Mesoaxial synostotic syndactyly with phalangeal reduction to be allelic disorders

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Camptosynpolydactyly is a rare and complex type of hand malformation that was first reported in a child and subsequently in fetus from Muslim family in India. The proband had disorganized hands consisting of polydactyly with 2 digits arising from the dorsum of the hands. In addition there was syn- and camptodactyly of some fingers, syndactyly of toes and nails were dysplastic. There were no other anomalies present. Subsequent fetus showed similar anomaly and was terminated.
Homozygosity mapping and exome sequencing was done in proband after obtaining informed consent. The sequencing reads were mapped, annotated and filtered for known polymorphisms and synonymous variants. The patient revealed two consequent homozygous substitution mutations (c.[220G>T;221A>T]) in Basic Helix Loop Helix (BHLHA9) gene. These mutations result in substitution of Glutamic acid at 74 position with Leucine (p.E74L). The mutation was confirmed by sanger sequencing in parents and sibling and was predicted to be 'disease causing' by mutation prediction software.
BHLHA9 has already been reported to be involved in embryonic limb development. Duplications of 17p13.3 region involving BHLHA9 gene are known to be associated with Split hand/foot malformation with long bone deficiency (SHFLD3;612676). Recently missense mutations in BHLHA9 gene were identified in Mesoaxial synostotic syndactyly with phalangeal reduction (MSSD;609432). The phenotype of patients with MSSD is much milder compared to our patient. Mutations in MSSD have been reported at amino acid numbers 71, 73 and 75. Our patient had mutation at position 74. The reason for the difference in phenotype is not clear but it appears that both MSSD and camptosynpolydactyly are allelic disorders.
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PS11.025

Clinical Genetics in the Dutch West Indies: 5-years' experience of clinical and molecular analysis of 350 patients with congenital malformation disorders and intellectual deficit.

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The islands Curacao, Aruba, St. Maarten and the three BES-islands (Bonaire, St. Eustatius, and Saba) are part of the Kingdom of the Netherlands. Their total population of approximately 300.000 consists of a mixture of different races, which makes them very interesting for genetic analysis. Until recently Clinical Genetics was not part of regular pediatric care on these Dutch Caribbean Islands. Since 2010, clinical genetic outpatient clinics have been established at the pediatric departments of St. Elisabeth Hospital, Curaçao, Dr. Horacio Oduber Hospital, Aruba, Fundashon Mariadal, Bonaire, and St.Maarten Medical Center, St. Maarten.

Here we present the clinical and molecular genetic results of the first 350 consultations. The majority of cases presented with intellectual deficit and/or congenital malformations. A clinical and molecular diagnosis could be established in approximately 40% of the cases.

The first results of an Aruban cardio-genetics cohort showed a high incidence of 12.3/1000 live births with congenital heart diseases. Genome wide analysis revealed copy number variations (CNVs) that might be associated with the clinical phenotypes. In addition we found a significant number of patients with at least one region of homozygosity larger than 10Mb, suggestive for identity by descent or consanguinity. Analysis of homozygous regions is currently pending to identify candidate genes that might explain the congenital heart disease phenotype in patients without a molecular diagnosis.

Since the incidence of certain congenital anomalies is higher than the global incidence, and patients come from relatively closed island communities with very detailed phenotype description, these cohorts are very interesting for future genetics studies.

PM11.026

Novel mutations in IGF1R and SYNM cause reccessive congenital diaphragmtic hernia and mental retardation

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Autosomal recessive congenital diaphragmatic hernia (CDH) with mental retardation, IUGR, hearing loss, short stature, failure to thrive (FTT) and dysmorphism was diagnosed in three individuals of an Arab Israeli family. MRI demonstrated hypomyelination with narrowing of corpus callosum. Homozygosity mapping yielded 4 homozygous regions and whole exome sequencing of an affected individual identified only two mutations in these regions, both of which within the same 15q26.3 locus: IGF1R (NM_000857.3:c.1915G>A, p.639G>S) and SYNM (NM_145728.2:c.1859G>T, p.620L>F). None of the mutations were found in 100 ethnically matched controls and both mutations fully segregated as expected within the studied family.

Deletion mutations in this chromosomal locus, harboring both IGF1R and SYNM, have been previously linked with CDH. Mutations in IGF1R have been demonstrated to cause hearing loss, IUGR, short stature and FTT. Interestingly, null mutation of DESMIN, direct interactor of SYNM, leads to diaphragmatic hernia in adult mice. We thus present a complex phenotype, presumably caused by homozygous mutations in two genes which are in linkage disequilibrium. We speculate that the IGF1R mutation is the cause for FTT, IUGR, hearing loss and short stature and that the SYNM mutation results in the CDH.

PS11.027

De novo heterozygous mutations in SMC3 cause a range of Cornelia de Lange Syndrome-overlapping phenotypes

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Cornelia de Lange syndrome (CdLS) is an inherited disorder characterized by facial dysmorphism, growth and cognitive impairment, limb malformations and multiple organ involvement. Mutations in five genes, encoding subunits of the cohesin complex (SMC1A, SMC3, RAD21) and its regulators (NIPBL, HDAC8), account for 70% of patients with CdLS or overlapping phenotype.

Here, we present clinical description of seventeen patients with CdLS-like features caused by mutations in SMC3 and assess the degree of overlap with typical CdLS phenotype. Of these, ten probands are novel and seven individuals have been previously reported. Furthermore, we mapped all mutations to the known structure of the SMC complex to predict functional consequences.

All patients exhibited clinical findings of overlap with typical CdLS patients, in particular harboring SMC1A mutations. Although SMC3-associated phenotypes are also characterized by postnatal microcephaly but with a less distinctive craniofacial appearance, a milder prenatal growth retardation that worsens in childhood, few congenital heart defects and an absence of limb deficiencies.

Likewise some functional indications previously reported, our modelling of the mutation effects support the hypothesis that the mechanism of pathogenicity in SMC1A and SMC3 related CdLS might be due to a dominant negative effect of the altered protein resulting from missense or in-frame mutations. Nevertheless, the finding of one truncating SMC3 mutation, leads us to consider haploinsufficiency as additional potential cause of pathogenesis.

This work confirms that SMC3 mutations account for ~1-2% of CdLS-like phenotypes and emphasizes the importance of SMC3 mutation screening, which will allow us to better assess for genotype-phenotype correlation.

PS11.029

FISHing for a tissue-specific mosaic monosomy of chromosome 21

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Non-mosaic chromosome 21 monosomy is considered incompatible with life. Here, we present an exception, which turned out to be an example of tissue-specific mosaic monosomy of chromosome 21. In a 9-month-old girl with severe congenital malformations, cytogenetic analysis of blood revealed monosomy of chromosome 21. Array CGH (molecular karyotyping) has confirmed the loss of chromosome 21. To determine the background of viability in the index case, FISH analysis of different tissues (blood and buccal cells) was done using DNA probes for 21q22.13q22.2 and D13Z1/D21Z1 (pericentromeric DNA) loci and multicoulor banding. In buccal cells, we have detected ring chromosome 21 was detected in 80% of cells. The region of chromosome 21 lost through the ring chromosome formation was estimated as 21q22.11qter. The patient presented with microcephaly, large low-set ears, glaucoma, perforated corneal ulcer, protruding frontal suture, skull asymmetry, short neck, arachnodactyly, pectus excavatum, ventriculomegaly and thrombocytopenia. The present case appear to be one of few cases of mosaic chromosome 21 monosomy and probably one of the first case of tissue-specific mosaicism for chromosome 21 loss more-or-less well documented. One can speculate that this case is the result of intercellular natural selection between normal and abnormal cells as proposed earlier (Yurov et al., 2007, 2009, 2010). Interestingly, it is the FISH-based techniques that have helped to rule out the presence of normal cells. Thus, FISH and array CGH should be applied to analyze different tissues to diagnose similar cases.

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PM11.030

The ciliopathy protein CC2D2A associates with NINL and plays a role in RAB8A-MICAL3 regulated vesicle trafficking

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Ciliopathies are a genetically and phenotypically heterogeneous group of human developmental disorders caused by dysfunction of primary cilia. Joubert syndrome (JS) is characterized by a distinctive hindbrain malformation variably associated with retinal dystrophy and other ciliopathy phenotypes. Mutations in CC2D2A, encoding a protein localized at the ciliary transition

zone, are found in ~10% of patients with JS. The ciliary transition zone plays an important role in controlling the ciliary protein content, but the precise mechanism remains unclear. Previous studies in the zebrafish demonstrated a role for Cc2d2a in Rab8a-dependent vesicle trafficking in photoreceptor cells. In this work, we identify the centrosomal protein NINL as a physical interaction partner of CC2D2A through a yeast-2-hybrid screen. NINL partially co-localizes with CC2D2A at the base of cilia and ninl knockdown in zebrafish leads to photoreceptor outer segment loss, mislocalization of opsins and vesicle accumulation, similar to cc2d2a-/- phenotypes. Moreover, partial ninl knockdown in cc2d2a-/- embryos enhances the retinal phenotype of the mutants, indicating a genetic interaction in vivo, for which an illustration is found in patients from a JS cohort. Similar to zebrafish cc2d2a mutants, ninl morphants display altered Rab8a localization. Further exploration of the NINL-associated interactome identifies MICAL3, a protein known to interact with Rab8a and to play an important role in vesicle docking and fusion. Together, these data support a model where CC2D2A associates with NINL to provide a docking point for cilia-directed cargo vesicles, providing a mechanism by which transition zone proteins can control the protein content of the ciliary compartment.

PS11.031

A specific R391X mutation in the RUNX2 gene may be associated with hearing loss in the Cleidocranial Dysplasia: Analysis of four families

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Cleidocranial dysplasia (CCD, MIM119600) is an autosomal dominant skeletal dysplasia characterized by delayed closure of the cranial sutures, aplasia or hypoplasia of the clavicles and dental abnormalities. These findings accompanied by mobility of droopy shoulders, frontal and parietal bossing, hypertelorism, brachycephaly, presence of multiple wormian bones, wide pubic symphysis, supernumerary, late erupting teeth and short stature. CCD can be diagnosed by clinical and radiological evaluation and validated by molecular studies. Heterozygous loss of function RUNX2 gene, which plays an important role in osteogenesis and differentiation of precursor cells, causes CCD phenotype.

We report six cases from four unrelated families with CCD phenotype. First family had a classic CCD phenotype and, IVS4+4delAAGT mutation was detected. Second family had also similar clinical findings with first family and molecular analysis of RUNX2 is not completed yet. Third family also had classic CCD phenotype and accompanied by hypothyroidism and mixed type hearing loss. Her father was presented CCD phenotype and mild sensorineural deafness. Mutation analysis of third family revealed R391X in the RUNX2 gene. Last case had also classic CCD phenotype and molecular analysis of RUNX2 isn't completed yet. In CCD patients, though hearing loss was identified in the literature, previously, there is no comprehensive data about auditory capacity of patients who had R391X mutation in the RUNX2. Our data suggest that these specific mutation may cause conductive and/or sensorineural hearing loss in CCD. To elucidate this hypothesis further functional analysis should be performed.

PM11.032

Clinical exome sequencing emerges as an effective diagnostic tool in Saudi pediatric patients with suspected genetic etiology: a King Fahad Medical City (KFMC) experience

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We present here, for the first time, an observational study of 69 Saudi patients seen by clinical geneticists who ordered the clinical exome sequencing (CES) tests at KFMC. All the patients were pediatric; 33 females (48%), 36 males (52%); mean age for females, 5.6±0.6 and for males 4.6±0.5 years. The patients presented with diverse clinical indications; intellectual disability (ID: 65%, 45/69), mitochondrial disease (10%, 7/69), multiple congenital anomalies (MCA: ~6%, 4/69), metabolic disorders (~4%, 3/69) and others comprising of individual cases (~15%, 10/69). We analyzed the CES data with respect to the phenotypic indications at initial diagnoses, overall diagnostic yield, mode of inheritance, the spectrum of genetic mutations, consanguinity and the incidental findings reported. We observed an overall diagnostic rate of 42% (29 of 69 cases; 95% CI-40%-44%) with 48% (14 of 29) of the mutations reported as variants of unknown clinical significance but likely pathogenic and 52% (15 of 29) as known disease causing genetic alterations. The rate of molecular diagnosis was 44% (20/45) for ID, 57% (4/7) for mitochondrial disorders, 33% (1/3) for metabolic disorders, 25% (1/4) for MCA. Mendelian inheritance patterns included 85% (23/29) autosomal recessive (AR), 3.7% (1/29) autosomal dominant (AD) and 7.4% (2/29) X-linked. Interestingly, the rate of consanguinity among positive cases was around 80%. One patient with MCA without ID and one with movement disorder (2/29, 6.8%) received molecular diagnoses of two overlapping AR genetic disorders.

In summary, clinical exome sequencing is emerging as a potential molecular diagnosis tool in Saudi pediatric patients suspected with genetic etiology with a diagnostic rate of 42%.

PS11.033

A case of Cockayne syndrome detected by chromosomal microarray analysis

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Chromosomal microarray analysis (CMA) is widely used for search of chromosomal microdeletions/microduplications but in some instances is helpful for detection of monogenic disorders. Our case of Cockayne syndrome, type A (CSA), rare autosomal recessive disorder produced by ERCC8 mutations, is an interesting example. The 8-year-old girl, an only child of non-consanguineous Russian parents originating from different regions, had pronounced delay of motor and especially of mental development, growth deficiency (100 cm, 13 kg), microcephaly (42 cm), typical face with deep-set eyes, early hypohidrosis, ataxia, demyelinating polyneuropathy with feet deformity, atrophy and multiple small calcinates on brain MRI, and optic atrophy but no skin photosensitivity, deafness, retinal degeneration or cataract. Mutations in ERCC6 gene responsible for more severe CSB were not found. DNA test for CSA, which is not performed in our centre routinely, was planned. Meanwhile, CMA (Affymetrix CytoScan HD Array) was carried out in search of other possible diagnoses, and homozygous deletion of 5q12.1 was detected. Molecular karyotype: arr[hg19] 5q12.1(60,131,474-60,192,457) x0. The deletion had size 60983 bp and encompassed two genes, ELOVL7 (of no clinical significance) and ERCC8. Moreover, an increased number of long contiguous stretch of homozygosity (LCSH) - 14% of genome - was found. Both CMA findings are also strongly unusual for apparently non-inbred family. Homozygous deletion was confirmed by ALFP analysis of ERCC8 exon 12. Verified CSA diagnosis permits prenatal or preimplantation testing in the family and proper supportive medical care for the patient minding risk of immunological and other complications.

PM11.034

Cervical spinal cord compression caused by calcification of the yellow ligament in Coffin-Lowry syndrome

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Coffin-Lowry syndrome (CLS) is an X-linked mental retardation syndrome characterized by severe to profound intellectual disability and musculoskeletal manifestations. Calcification of the yellow ligament (CYL) in early adulthood, resulting in myelopathy by spinal cord compression, had been previously reported in 3 CLS patients of Japanese origin. Here, we report on an additional 3 CLS patients with CYL: a 12-year-old boy and a pair of 17-year-old monozygotic twins. Ossification of the posterior longitudinal ligament and of the yellow ligament both represents ectopic ossification in the cervical and thoracic spine region. This ossification is a common disorder among Japanese and other Asian populations, resulting in compressive meylopathy and/radiculopathy in patients. Loss-of-function mutations in RSK2, a protein required for osteoblast differentiation and function, lead to CLS and are responsible for the skeletal abnormalities observed in CLS patients. Together with these results, the condition of CLS is likely accelerating the development of calcification in the ligaments and fascicles in our patients. Early detection and survey of this serious complication should be considered in the management of CLS, especially for patients of Oriental origin.

PS11.035

Insulin response dysregulation explains abnormal fat storage and high risks of diabetes mellitus type 2 in Cohen Syndrome

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Cohen Syndrome (CS) is a rare autosomal recessive disorder, with glycosylation defect secondary to mutations in VPS13B gene, which encodes a protein of the Golgi apparatus. Besides congenital neutropenia, retinopathy and intellectual deficiency, CS patients are faced to obesity. Metabolism investigati-

ons show abnormal glucose tolerance tests and HDL values in some patients that should be risk factors for the development of diabetes mellitus and/or cardiovascular complications. To understand the mechanisms involved in CS fat storage, we used two models of adipogenesis differentiation: (i) SGBS preadipocytes with VPS13B invalidation thanks to siRNA delivery and (ii) CS primary fibroblasts. In both models, VPS13B invalidation leads to an accelerated differentiation into fat cells, which is confirmed by an earlier and an increased expression of specific adipogenic genes, consequently to an increase response of cells to insulin stimulation. At the end of the differentiation protocol, these fat cells exhibit a decreased in AKT2 phosphorylation after insulin stimulation, in favor of insulin resistance. Thus, in association with the in-depth analysis of the metabolic status of the patients, this study allowed us to recommend an appropriate nutrition education in order to prevent the occurrence of diabetes mellitus as well as recommendation for follow-up of CS patients, in particular for metabolic syndrome development. We also preconize not to use the term obesity in CS, but abnormal fat repartition, which should limit the number of patients whom are addressed for CS diagnosis only on the basis of intellectual deficiency associated with obesity.

PM11.036

Complex chromosomal rearrangement - trisomy 9p24.3q21.11, duplication 17q25.3, deletion 19p12 - in a child with severe hypotonia, facial dysmorphism and genital hypoplasia

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The male child was born spontaneously as the second child of healthy non-consanguineous parents after 38 weeks of gestation after an uneventful pregnancy. Postpartal hypotonia, reduced muscle reflexes and weak crying were the first suspicious symptoms. Further clinical investigations revealed rhizomelia, craniofacial dysmorphism such as frontal bossing, flat occiput, flat nasal bridge, hypertelorism, epicantal folds, deep-set, simple-shaped ears and micro-retrognatia. Furthermore a simian crease, deep set thumbs, absent distal crease of the fifth digits, sacral dimple, skintag in the middle of the thorax and genital hypoplasia with cryptorchidism were evident. In the neonatal period a non-alloimmun-thrombopenia appeared that revovered spontaneously.
In the age of 5 months, the suckling was presented to the genetic counceller for the first time. Muscle hypotonia was still evident and presented as poor headcontrol and reduced spontaneous movement. However the baby could laugh and was alert. There was a tendency for infections. The family history was unremarkable, without evidence of abortions and disabilities.
Conventional chromosomal analysis revealed an additional derivative chromosome 9. Comparative genomic hybridization showed a complex chromosomal rearrangement with a terminal duplication 17q25.3 as well as a deletion 19p12 in addition to the trisomy 9p24.3q21.11. Using FISH analysis, the derivative chromosome 9 could be characterized as a product of a reciprocal translocation 9 and 17 with the correct karyotype 47,XY,+der(9)t(9;17)(q21.11;q25.3).
According to literature the patient's main symptoms are in compliance with trisomy 9 syndrome. This case again demonstrates that array CGH can reveal a complex chromosomal aberration even after a clear-cut conventional cytogenetic result.

PS11.037

Complex chromosome imbalances in form of monosomies 6p12.3-p21.1 and 18p11.21pter together with trisomy 19q13.41qter

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Introduction: A combination of several different approaches, including routine karyotype, FISH, and CGH studies are useful in identifying genes and regions responsible for phenotype modifications.
Material and Methods: In 23-years-old male using GTG and RBG techniques a monosomy of the short arm of chromosome 18p11.21→pter has been found in his karyotype. Array comparative genomic hybridization, was prepared using **SurePrint G3 Human CGH Microarray Kit, 8x60K. Results were analysed using Agilent CytoGenomics 2.7 Software.** All of the rearrangements were confirmed by FISH studies.
Results: We detected loss of 6449 kb at short arm of chromosome 6, loss of 13 687 kb at short arm of chromosome 18 and gain of 7138 kb at long arm of chromosome 19. Morphological phenotype of boy consist of short stature, long, lumpy head, slightly sunken temples area, sloping forehead, high, diffuse frontal hairline, triangular face, prominent eyebrows, ptosis, hypertelorism, flat nasal bridge, the long ridge and thick tip of the nose, big, raised to the top nostrils, flanged out lower lip, short nasolabial distance, broad mouth fissure, ear lobes obliquely backward, crowded, misaligned teeth. In addition the dystonia and hearing problems, intellectual disability and delay of speech development were observed.
Conclusion: Two additional sub-microscopic changes in form microdeletion 6p12.3-p21.1 and microduplication 19q13.41→qter as a highly complex chromosomal rearrangement involving three chromosomes together with deletion 18p11.21→pter resulted in complex phenotype in boy with previously diagnosed like de Grouchy syndrome.
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PM11.038

Associated malformations in cases with congenital diaphragmatic hernia

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The etiology of congenital diaphragmatic hernia (CDH) is unclear and its pathogenesis is controversial. Because previous reports have inconsistently noted the type and frequency of malformations associated with CDH, we assessed these associated malformations ascertained between 1979 and 2007 in 386,088 consecutive births. Of the 139 patients with the most common type of CDH , the posterolateral, or Bochdalek-type hernia , 85 (61.2%) had associated malformations. These included: chromosomal abnormalities (n=25, 18.0%including 12 cases with trisomy 18); non-chromosomal syndromes (Fryns syndrome, fetal alcohol syndrome, De Lange syndrome, CHARGE syndrome, Fraser syndrome, Goldenhar syndrome, Smith-Lemli-Opitz syndrome, multiple pterygium syndrome, Noonan syndrome, spondylocostal dysostosis, and Beckwith-Wiedemann syndrome); malformation sequences (laterality sequence, ectopia cordis); malformation complexes (limb body wall complex) and non syndromic multiple congenital anomalies (MCA) (n=36, 25.9%). Malformations of the cardiovascular system (n=53, 27.5%), urogenital system (n=34, 17.6%), musculoskeletal system (n=29, 15.0%), and central nervous system (n=19, 9.8%) were the most common other congenital malformations. We observed specific patterns of malformations associated with CDH which emphasizes the need to evaluate all patients with CDH for possible associated malformations. Geneticists and pediatricians should be aware that the malformations associated with CDH can often be classified into a recognizable malformation syndrome or pattern (35.3%).

PS11.039

Unusual form of congenital ichthyosiform erythroderma (CIE) in two siblings of an Austrian family. Attempts to unravel genetic basis of this disorder.

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Congenital ichthyosis is a rare disease and particular subtypes like lamellar ichthyosis (LI) have a very low incidence of less than 1:500.000. In a couple of Austrian origin where a distant relationship of both parents was likely because their grandparents were born in the same small village, two of their four offsprings were affected by CIE/LI. The so-called collodium baby phenotype was present in one daughter and one son, whereas the other two offsprings are healthy. The affected girl died with 3 month due to disease related complications and the affected son is now 28 years old. He showed early onset of rickets not recognized before an age of 16. Because sufficient vitamin D treatments started late, he suffered from multiple fractures and developed bowing of upper and lower limbs. Growth retardation (150 cm length) and microcephaly (OFC 49 cm) but normal mental development and hearing were found. Some of his progeroid features and light sensitivity resemble similarities with Trichthiodystrophy, another heterogenic autosomal recessive disorder, caused by helicase subunits of transcription/repair-vector-gene mutations. Homozygosity mapping was performed by SNP array analysis using Affymetrix CytoScan 750 with analysis suite 2.1.0.16. Results obtained with patients DNA clearly support parental consanguinity. Just 3 homozygous segments ranging from 18,4 Mb at 5q23.2 from PHAX- to FGF1-gene and 4.7 Mb at 1q21.1 and 3.4 Mb at 12q24.11 were identified. Since none of thus far reported genes for CIE or LI map to these genomic segments, focused NGS approaches to identified potential candidate genes are now implemented.

PM11.040

Exome Sequencing As A Useful Tool To Correctly Diagnose A Congenital Myopathy In Two Sibs With An Unclear Phenotype And A Tentative Diagnosis Of Opitz-C Syndrome

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Opitz C Syndrome (OTCS) is an ultra-rare disease with less than 60 patients diagnosed worldwide and a broad range of affectation, making its diagnosis very challenging. The OTCS gene/s has not been identified, yet. We recruited a cohort of 14 patients diagnosed as OTCS or C-like syndrome, some with a "tentative" diagnosis. To search for the OTCS gene, 4 patients and their parents, including two brothers with an unclear phenotype, were subjected to whole-exome sequencing (WES). While the WES sequences were being analyzed, a thorough clinical study of the older brother, including a muscle NMR, strongly suggested a congenital myopathy, rather than OTCS.
The WES analysis revealed that the 2 brothers bore 2 mutations in the RYR1 gene, inherited in an autosomal recessive manner. The maternal mutation was a missense change (p.Cys489Phe), while the paternal was a nonsense mutation (p.Arg2241STOP) previously associated with congenital myopathy and atypical periodic paralysis. The p.Cys489Phe mutation is predicted to be severely damaging by SIFT, PROVEAN and PolyPhen2. In addition, the Cysteine 489 residue is conserved among 33 vertebrate species and the nature of the amino acidic change and its position in a well-defined alpha helix suggest that the correct folding of the RYR1 protein may be affected. However, further functional studies need to be performed to demonstrate its pathogenicity.
WES is becoming a powerful tool in the molecular diagnosis of patients with unclear phenotypes, pathologies with an unknown molecular basis or in which very large genes (such as RYR1 with 106 exons) are involved.

PS11.041

Beyond Cohesinopathy: Mutations in chromatin-associated factors as genetic cause of CdLS-overlapping phenotypes

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Cornelia de Lange syndrome (CdLS) is a genetically heterogeneous disorder manifesting extensive phenotypic variability. Mutations in NIPBL, SMC1A, SMC3, RAD21 and HDAC8, encoding subunits or regulators of cohesin, are found in about 70% of the patients.
Next generation sequencing approaches have allowed us to identify heterozygous de-novo mutations in five patients with CdLS phenotypes who were previously negative for mutations in the CdLS genes.
All five mutations alter chromatin-associated factors and each is predicted to result in a loss of function. Two mutations, one nonsense and one 4-bp frame-shift deletion, were found in the ANKRD11 gene, that is associated with KBG syndrome. The three other mutations disrupt components of the SWI/SNF chromatin-remodeling complex, previously associated with Coffin-Siris and Nicolaides-Baraitser syndromes. These mutations include a missense substitution in SMARCB1, a frame-shift deletion in ARID1A and a chromosome microdeletion including the ARID1B gene.
Our results support recent molecular findings that describe an intimate link between cohesin and the SWI/SNF complex in regulating transcription. Furthermore, ANKRD11 associates with histone deacetylases to enable its role in transcriptional regulation. These findings add to the growing body of work supporting the hypothesis that CdLS and overlapping phenotypes result from alterations in specific gene expression patterns.
In summary, mutations in ANKRD11 and components of the SWI/SNF complex can result in clinical pictures that are difficult to distinguish from CdLS and related disorders. Thus, sequence analysis of these genes should be strongly considered for those patients with suspected clinical diagnoses of CdLS who were negative for CdLS-associated genes.

PM11.042

Acute Megakaryoblastic leukemia in a patient with Cornelia de Lange syndrome. Could germline mutations in cohesin predispose to cancer ?

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Cornelia de Lange Syndrome (CdLS) is a rare disorder characterized by a distinctive facial dysmorphism associated with growth delay, microcephaly, mental retardation and limb anomalies. CdLS is caused by heterozygous mutations in genes encoding the core proteins of the cohesin complex or its regulators. Cancer genomics analyses have discovered a very high mutation rate in Down syndrome-associated acute megakaryoblastic leukemia (DS-AMKL) suggesting that cohesin mutations may be the third genetic hit responsible for AMKL. Despite the potential role of cohesin in malignancy, increased tumor incidence in CdLS patients has not been described.
We report the occurrence of AMKL in a 3 years boy with a clinical diagnosis of CdLS. Leukemic cells displayed the same morphologic and immunophenotypic characteristics found in DS-AMKL blasts. Molecular and cytogenetic analysis revealed a frameshift mutation in *GATA1* and trisomy 21 in the bone marrow blast cells. Analysis of a blood sample of the patient at the age of 3 weeks by exome sequencing identified a *NIPBL* mutation responsible for the CdLS but also revealed the presence, at a mosaic level, of the *GATA1* mutation and trisomy 21 confirming that these defects which are primary events in DS-AMKL are here, secondary events.
This is the first report of leukemia in a patient with CdLS. The clinical implication of this observation needs further investigations to determine whether cohesin mutations in CdLS patients may predispose them to cancer. Our findings suggest that cohesin defects, in addition to their known role in leukemic progression, may also represent the first genetic hit to initiate leukaemogenesis by inducing aneuploïdy.

PS11.043

A series of 38 novel germline and somatic mutations of NIPBL in Cornelia de Lange syndrome.

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Introduction: Cornelia de Lange syndrome is a rare and severe multisystemic developmental disorder. Five genes have been identified so far, all involved in the cohesin complex. NIPBL represents about 60% of identified heterozygous mutations. The majority of cases results from a de novo event. Recently, NIPBL somatic mosaicism has been highlighted through buccal cell DNA study in some patients with a negative molecular analysis on leukocyte DNA.
Materials and Methods: Here, we present a series of 38 patients with a Cornelia de Lange syndrome related to an heterozygous NIPBL mutation identified by Sanger sequencing. The diagnosis was based on the following criteria: 1) intrauterine growth retardation and postnatal short stature, 2) feeding difficulties and/or gastro-oesophageal reflux, 3) microcephaly, 4) intellectual disability and 5) characteristic facial features.
Results: We identified 37 novel NIPBL mutations including 34 in leukocy-

tes and three in buccal cells only. All mutations shown to have arisen de novo when parent blood samples were available. As previously reported, the present series confirms the difficulty in predicting the phenotype according to the NIPBL mutation. Until now, somatic mosaicism has been observed for thirteen cases which does not seem to be consistently associated with a milder phenotype. Besides, several reports support a postzygotic event for those cases.<

Conclusion: Considering these elements, we recommend a first-line buccal cell DNA analysis in order to improve gene testing sensitivity in Cornelia de Lange syndrome and genetic counseling.

PM11.044

Corpus callosum anomalies in fetuses: from fetal pathology to NGS and reverse phenotyping

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Corpus callosum (CC) is the major brain commissure connecting the homologous areas of both hemispheres at the midline. CC malformations (CCM) are the most frequent brain malformations with an incidence of 1/4000 newborn often associated with chromosomal anomalies or mendelian syndromes with recessive and dominant inheritance. Recurrence is observed in 5 % of cases. Children with CCM have an uncertain neuro-developmental outcome. Therefore, counseling remains challenging, especially prenatally.

We systematically reviewed the data of 142 fetuses with CCM as isolated or associated autopsy findings in our center. We first completed the cytogenetic analysis by a CGH array when the underlying etiology was not found: 108 (76%) of cases remained unsolved.

In our effort to identify the disease causing gene of CCM antenatally, we used exome sequencing in 10 trios and a targeted high throughput sequencing strategy including 423 genes in 64 fetuses and 32 are ongoing. NGS allowed several diagnosis, such as a PDH deficiency (PDHA1), PCH with ACC (AMPD2), genitopatelar (KAT6B), Primrose (ZBTB20), Coffin-Siris (ARID1A and ARID1B) or Chudley Mac Cullough syndrome (GPSM2). Interestingly some diagnosis were not possible antenatally due to the absence of specific signs, but reevaluation of fetalpathological data (reverse phenotyping) allowed to support NGS findings. These situations will be illustrated.

All together, to date, as analysis are still ongoing, combined fetal imaging, fetal necropsy, cytogenetic and molecular analysis allowed the identification of the cause in at least 30 % of fetuses: 15% chromosomal anomaly and 15 % of mendelian disorder. The necessary reverse phenotyping underlines the importance of fetal necropsy following pregnancy terminations for CCM.

PS11.045

Whole exome sequencing for craniofacial anomalies: the NIjmegen experience

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Introduction: Whole exome sequencing (WES) is a successful genomewide approach to identify genetic causes of heterogeneous diseases. Our department was among the first to implement exome sequencing in clinical genetic diagnostics. We show the results of WES for diagnosis of craniofacial anomalies. Methods: Twenty patients with craniofacial anomalies (familial or syndromic cleft lip/palate, oligodontia and craniosynostosis) were investigated by means of WES. WES was performed on an Illumina HiSeq2000TM platform after enrichment with the Agilent SureSelect XT Human All Exon 50 Mb kit. A two-step analysis was used in which a CFA gene panel containing 94 genes, based on the OMIM database and recent publications, was analysed first before opening the whole exome. Results: In the first step analysis, a genetic diagnosis was made in 5/20 index patients (25%). These comprised mutations in *COL11A2* causing Stickler syndrome, in *GLHR3* causing van der Woude syndrome type 2, and *WNT10A* mutations in oligodontia patients. Whole exome analysis in 11/15 patients diagnosed Kallmann syndrome, caused by a *FGF8* mutation, in a family presenting with cleft lip/palate. Conclusion: With a diagnostic yield of 25% in the first analysis these results of the WES based CFA gene panel in a small patient cohort are promising. Having a genetic diagnosis allows optimal management and adequate genetic counselling. Therefore we recommend that diagnostic WES should be offered to all patients with syndromic or familial forms of orofacial clefting and oligodontia.

PM11.046

Partial monosomy 5p and partial trisomy 13q in a patient with cat-like cry and postaxial polidactily detected by SNP array - a case report

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Here we present a case of a chromosomal rearrangement in a 7-month old boy from Georgia with partial deletion of 5p and partial duplication of 13q with features of both Cri-du-Cat (CdC) and trisomy 13. The patient was born from a second pregnancy of non-consanguineous parents on the 37th weeks of gestation. The first pregnancy was terminated due to multiple congenital anomalies. At birth the patient weighted 2450 gr, his lenght was 46 sm, OFC - 33 sm and presented with cat-like cry. He also has hypotonia, stridor, failure to thrive, postaxial polydactily, unilateral inguinal hernia, epilepsy/seizures and dysmorphic face: micrognatia, scaphocepaly, malformed low-set ears with posterior rotation, low nasal bridge, hypertelorism, thin upper and thin lower lips, long philtrum, high-arched palate. The SNP array revealed a deletion of 23Mb in 5pter (del 5p15.33-p14.3) and a duplication of 54Mb genetic materials in 13qter (dup 13q21.2-q34), that was inherited from a balanced translocation carrier mother. Only several cases of his type of chromosomal rearrangement have been so far. Our case further confirms the involvement of critical region 5p15.2 for cat-like-cry and involvement of GPC5 and GPC6 genes located on chromosome 13 (13q31.3q32.1 region) for postaxial polydactily.

PS11.047

Reverse phenotyping of a patient with CRIPT gene mutation and further delineation of the associated phenotype

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We report on a 3 ½ year old boy, with prenatal onset growth deficiency (height:-4SD), microcephaly (OFC:-3.5 SD), transient neonatal pancytopenia, facial dysmorphism, feeding difficulties, developmental and speech delay, global hyperlaxity, significant sleep disturbance, and genital, ocular and extremities anomalies. He also presents with generalized pigmentation anomalies, and signs of ectodermal dysplasia. Array CGH (Agilent 60k), cytogenetic diagnosis of chromosomal breakage syndrome and metabolic screening are negative. The whole exome sequencing performed revealed a homozygous frame-shift mutation of the CRIPT gene, recently described as a novel primordial dwarfism gene (Shaheen et al, 2014). The mutation (c.132delA), described as probably pathogenic, was confirmed in the homozygous state by Sanger sequencing. Both healthy consanguineous parents were proven to be carrier in the heterozygous state.

Few available clinical data of the 2 described patients show very similar clinical appearance with strikingly facial dysmorphism, growth deficiency, microcephaly, psychomotor delay, and ocular and extremities anomalies. Mottled hypopigmentation is also described in the older patient.

This report is an example of "reverse phenotyping". The first description of the CRIPT gene by Shaheen et al helped us to reach a diagnosis in our patient. Nevertheless the term of primordial dwarfism and its broad definition used by the authors can be confusing; and can prevent some clinicians from suggesting this diagnosis. Cutaneous signs seem also to be very specific, and need to be precisely looked at in additional patients presenting with this unique syndrome.

PM11.048

Exome sequencing of sporadic patients with Currarino syndrome

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Currarino syndrome (CS) is a complex of congenital caudal anomalies including anorectal malformations, sacral defects and a presacral mass. CS is associated with mutations in the MNX1 gene in familial cases, but in sporadic cases less than one third of the patients have detectable MNX1 mutations. We looked for genetic causes of CS in sporadic patients by Whole Exome Sequencing.

3 trios were included in the study. In addition we included DNA extracted from the presacral mass from one patient, both directly from the biopsy and from cultivated fibroblasts derived from the sample. The library was sequenced on a HiSeq 2000 (Illumina) with 100 bp paired end reads. Exome capture was performed with the SureSelect XT Custom Human All Exon v5 Plus library (Agilent Technologies). In addition we added a custom made 6 MB whole genome regions around MNX1 on chromosome 7 to look for variations in regulatory elements.

The data were filtered with an in-house developed program (FILTUS) and registered variants from an in-house database were used to filter out normal variation. We looked especially for sequence variants in genes involved in proliferation, differentiation and embryogenesis. All models of inheritance were applied for candidate gene identification. The first approach was to filter the data through a candidate gene list. Second a de novo analysis was done. When no relevant genes were found, the next approach was to analyze the samples through other inheritance models. Some candidate genes will to be discussed and further investigated.

PS11.049

Cytogenomic investigation in 162 patients with multiple congenital abnormalities and developmental delay: Brazilian experience

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The genomic imbalances are the most common cause of multiple congenital abnormalities (MCA) and developmental delay (DD), however the etiology of these imbalances are not well understood, making difficult the counseling genetics and the treatment. Currently, the improvement of cytogenomics diagnostic techniques, such as the screening by arrays, is fundamental to set an unequivocal molecular diagnosis and a more suitable genotype-phenotype correlation for patients with MCA/DD.

We report our experience with the implementation of several array platforms (Affymetrix, Agilent and Illumina) and probe densities in clinical diagnostic and scientific research of patients without conclusive diagnostic. The results were confirmed by MLPA and/or FISH techniques.

Thus this study evaluated the genome of 162 patients with MCA/DD. All patients were previously assessed by conventional cytogenetic analysis. We identified several different genomic alterations in 137/162 (~84.6%) patients, including deletion, duplication and loss of heterozygosity. Some patients 41/137 (~30%) showed only one copy number variation in the genome, others 41/137 (~30%) presented two abnormalities and 55/137 (~40%) revealed three or more alterations on different chromosomes, maybe due to a complex rearrangements.

Our findings showed that the interpretation of genotype-phenotype correlations in patients with complex genomic rearrangements is a very difficult task but the results can directly contributes to the elucidation of new syndromes. The array is a powerful tool to identification and characterization of genomic abnormalities and provides accurate diagnosis of unidentified or unexplained diseases suspected to have a genetic cause, contributing to appropriate clinical management of the patients.

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PM11.050

Interstitial 1p32.3p32.1 deletion in a patient with multiple congenital anomalies

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Interstitial deletions of chromosome bands 1p32.3p32.1 are rare. Only nine unrelated patients with partially overlapping 1p32.3p32.1 deletions of variable size and position have been reported to date. We report on a 17-months-old boy with choanal atresia, hearing loss, urogenital anomalies and microcephaly in whom an interstitial deletion of 6.4 Mb was detected in 1p32.3p32.1 (genomic position chr1:54,668,618-61,113,264 according to GRCh37/hg19). The deleted region harbors 31 RefSeq genes. Notable genes are *PCSK9*, haploinsufficiency of which caused low LDL cholesterol plasma levels in the patient, and *DAB1*, which is a candidate gene for cognitive deficits and microcephaly. The patient broadens our knowledge of the clinical consequences of 1p32 deletions and facilitates karyotype-phenotype correlations. Additional patients with overlapping deletions and/or point mutations in genes of this region need to be identified to elucidate the role of individual genes for the complex clinical manifestations.

PS11.051

Deletion 2q31: clinical and molecular analysis based on a four new cases

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There are only few publications about interstitial deletions in long arm of 2 chromosome and only a few confirmed by array-CGH. Microarray technique allows to precisely define breakpoints which permits a more accurate phenotype-genotype correlation. On the one hand the phenotype of patients with 2q31 deletions seems to be well characterized but on the other hand the critical region for the phenotype is still unknown. The clinical manifestations are highly variable but the most common features include frequently observed fail to thrive, facial dysmorphism, developmental delay and mental retardation.

In our report we presents four new patients with de novo deletions overlapping 2q31. In all patients the breakpoints were characterized by a-CGH. The deletions ranged in size from 13 to 16Mb. In all patients microcephaly, high forehead, narrow mouth, small mandible, partial syndactyly of toes and sandal gap was stated. Additionally blepharophimosis, down slanting palpebral fissures, down-turned corners of the mouth and tapering fingers were present. One of the patients has an increased number of creases on the soles and palms which is one of the known features of wrinkly skin syndrome. In our report we try to delineate the phenotype of 2q interstitial deletions. We compare the phenotype of our patients with the ones presented in recent reports. We also suggest some genes candidates for the most common clinical manifestations of deletion in long arm of 2 chromosome.

PM11.052

A de novo 432 kb deletion in the 17q22 region: a case report and review of the literature

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Deletions involving the 17q22 region have been reported in the literature in nine patients. The size of the deletions reported to date is 8.18-1.98 Mb and a significant role of NOG and TBX has been suggested. Here we describe a girl with a 432 kb deletion in the 17q22 band without deletion of these genes. To our knowledge, this is the smallest reported deletion.

The patient was born at term by cesarean section because of breech position, birth measurements were normal. On physical examination at the age of 3 y, hypertelorism, upslanting and narrow palpebral fissures, epicanthal folds, short bulbous nose, long philtrum, tapered, short fingers, mild 5th finger clinodactyly, and proximally placed thumbs were observed. Developmental milestones were significantly delayed, and marked hypotonia was present. She had an atrial septal aneurysm, visual problems (astigmatism, hyperopia), and sensory processing disorder with a propensity for mutistic and aggressive behavior. Hypercalcemia with a tendency for lithiasis was diagnosed. A 432 kb deletion encompassing 12 genes in the 17q22 band was detected by aCGH analysis. The aberration was confirmed by FISH analysis as a de novo deletion.

Here we demonstrate the phenotype differences between our patient and the data from the literature and the possible influence of the deletion. The patients share some facial features and developmental delay. We suggest that in our patient, SUPT4H1 played an important role in development.
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PS11.053

Additional patient report with Delleman syndrome and lipoma: Oculo cerebro cutaneous syndrome (OCCS) or Encephalo cranio cutaneous lipomatosis (ECCL)?

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Delleman syndrome (or OCCS) is a very rare condition that has been reported in 30 unrelated patients t date. The diagnosis is based on triad of ocular (cyst, anophthalmia, microphthalmia, coloboma), cerebral (cerebral cysts, hydrocephaly, cortical atrophy or corpus callosum agnesis) and skin (focal dermal hypo- or aplasia, periorbital protruding outgrowths) anomaly. The differential diagnosis has to be made with ECCS and oculo-auriculo-vertebral spectrum (Goldenhar association). We report on the natural history of a full term boy, second child from caucasian unrelated healthy parents. Prenatal ultrasound diagnosed isolated ventricular dilation. Birth parameters include relative macrocephaly (weight P50, height: P50, OFC: P95). He presented tumour-like lesion on the right eyelid, bilateral periorbital skin appendages and skin hypoplasia with alopecia. Brain MRI showed left ventricular dilation, posterior midline shift to the right, cortical atrophy and extramedullary lipoma leading to spinal cord compression. Ophthalmology examination showed epibulbar dermoid, non-reactive pupils with bilateral pale optic disk. SNP array was normal (Agilent 44k). The patient was hospitalized at week 9 of life. He developed progressive neurologic demise and eventually died.
Phenotype in patients with OCCS may be highly variable. Some authors postulated on a continuum from ECCL to OCCS. In absence of any diagnostic marker, Alastair developed diagnostic criteria. In our patient, both syndromes fit as 'definite diagnosis'. The aetiology of OCCS remains so far unknown. Next generation sequencing (exome sequencing) could be the coming step to identify aetiology.

PM11.054

autosomal recessive developmental disorders

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Developmental disorders are a clinically and genetically heterogenous group of childhood conditions that involve serious impairment in specific developmental areas. To date, many types of developmental disorders have been recognised with a wide range of clinical manifestations and variable severities. Next-generation sequencing technologies have uncovered the genetic bases for a substantial number of these disorders yet many still of unknown causes.
In the DDD study, thousands of children exhibiting developmental disorders were recruited and investigated using the latest molecular testing methods to identify the underlying genetic defects. Among the first 1000 trios investigated in the study, two third of the diagnoses made were from de novo mutations. However, increasing the number of investigated trios has increased the power to detect and diagnose inherited conditions, including autosomal recessive disorders. The anticipated power of such big data is: 1) to enable the discovery of novel recessive genes, 2) to confirm the pathogenicity of new recessive genes with slight evidence in the literature, 3) to expand the phenotypic spectrum of known disease-causing genes, 4) to shed light on pathways that are disrupted in a group of affected children. For example, several unrelated patients in our dataset were carrying rare loss-of-function and non-synonymous biallelic-mutations in different genes encoding Glycosylphosphatidylinositol-anchored proteins. Two of these genes were recently identified and few patients have been reported in the literature. Therefore, our data endorse their pathogenicity and expand their clinical spectrums.
All this expected knowledge could most likely create new opportunities for genetic counselling, prevention, management and treatment of the disorders for the affected children and/or their families.

PS11.055

The Frequency Of Disorders Of Sex Development (DSD) And Novel Genetic Associations In Children With Neurodevelopment Disorders - Insights From The DDD Study

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Context: Collaborative project to review the phenotypic and genotypic data from children recruited to the UK wide DDD study.
Objective: To report the frequency and range of DSD phenotypes observed in DDD participants who have one or more associated 'neurodevelopmental delay' diagnostic Human Phenotype Ontology (HPO) term.
Methods: Retrospective review of anonymized data from participants in the DDD study.
Results: Of 7439 DDD participants recruited, 603 (8%) had at least one HPO term in the 'abnormalities of the genital system'. Of these 603 children, 370 (61%) had at least one 'neurodevelopmental delay' diagnosis with a total of 436 DSD phenotypes, the majority, 420 (94%) abnormalities of the external genitalia. Of the male external genitalia abnormalities, 212 (54%) were testicular, 74 (19%) were hypospadias, 57 (15%) were penile and 47 (12%) were other abnormalities. Testicular abnormalities included: unilateral cryptorchidism, bilateral cryptorchidisms, hydrocele and other phenotypes. Causative mutations were found in 14 DDG2P genes (https://decipher.sanger.ac.uk/), confirming a range of syndromic diagnoses with associated DSD, including: KBG syndrome, Meier-Gorlin syndrome, Alpha- thalassemia/mental retardation syndrome, Kabuki syndrome and Donnai-Barrow syndrome. Of these likely pathogenic mutations, 6 of 14 (43%) were found in DDG2P genes not previously associated with DSD.
Conclusions: A range of DSD phenotypes are found in patients with neurodevelopmental delay. Recognition of these associations should not be overlooked in the management of patients with complex conditions. Exomic sequencing through projects like DDD increases diagnostic yield whilst the identification of mutations in developmental genes may improve understanding about the pathogenesis of DSD.

PM11.056

A case of developmental delay and dysmorphic features with duplication 15q11q13 inherited from the mother

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A 10-year old boy with speech delay, dysmorphic features, short stature, joint hyperlaxity, muscular hypotony and conductive deafness is presented. His father was diagnosed with X-linked spondilopehyseal dysplasia tarda.
Birth parameters were normal. In the first months he developed motor developmental delay with muscular hypotony. At one year short stature was diagnosed. Repeatedly he has been treated for mild middle ear infections and a conductive hearing loss was diagnosed at 10 years. At this age (height 130,67 cm (-1.793 SDS); weight 28,25 kg (-1.194 SDS); head circumference 55,5 cm (0.478 SDS)) he presented with two cafe au lait spots. Several dysmorphic features were observed: long face, high vaulted palate, wide, retroverted nostrils, low set ears, tag on the lobule, deep philtrum, tented upper lip, joint hyperlaxity, proximal placed thumbs, wide spaced nipples and 2-3 toe syndactily of the both feet. The boy had difficulties in fine motor skills, concentration, attention and social skills. At the age of 11,5 years suspect of autism spectrum disorder was made.
aCGH detected interstitial duplication of approximately 6.1Mb at 15q11.2q13.1 (arr[hg19] 15q11.2q13.1(22,765,628-28,940,098)×3 mat), subsequently confirmed by FISH. Parental analyses identified the mother as a carrier of the same duplication.
Several reports have suggested that maternally transmitted 15q duplications were associated with autistic features with variable degrees of developmental delay, while paternally derived cases presented with no major medical problems. Genetic counseling is vital to discuss the recurrence risks and options for prenatal and preimplantation genetic diagnosis in familial cases of 15q11q13 duplication.

PS11.057

Unique familial 16q12.1-q22.1 duplication: clinical manifestations and cytogenetic analyses

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Background: Pure 16q duplications are rare chromosomal abnormality associated with dysmorphic features, intellectual disability, behavioral disorder and congenital anomalies. Even though the number of described cases is small, three main groups have been proposed: proximal (16q11-q13), proximal- intermediate (16q21-16q22), intermediate- distal (16.q23-q24) aberration, depending on the covered region of chromosme 16. Only 4 cases of the proximal- intermediate 16q duplication have been reported so far, with no familial cases described.
Report: We present a family of mother and two daughters, carrying duplication 16q12.1- q.22.1. Two-year-old girl was evaluated due to mild facial features and delayed milestones. Her one-year-old sister manifesting distinct dysmorphic phenotype, evident developmental delay, hypotonia and obesity was operated because of hypertrophic pyloric stenosis. Their mother displayed neither dysmorphia nor congenital defects but had moderate intellectual disability. High resolution karyotype of peripheral blood lymphocytes showed additional material on the long arm of chromosome 10. Array CGH analysis revealed duplication of 16q12.1- q22.1, estimated to be 16.54 Mb in size. Additional FISH analysis confirmed that duplicated material was of chromosome 16 origin in both sisters and mother.
Conclusion: The involved region contains 208 genes, some of which are known neurotransmitters, receptors or cell cycle regulators what is consistent with clinical findings. Limited available data and varying size of duplicated region does not allow for reliable genotype-phenotype correlation. Every newly described patient and family is a valuable contribution for a further delineation of phenotypic spectrum for those particular chromosomal regions.

PM11.058
Triallelic and epigenetic-like inheritance in human disorders of telomerase

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*Genetic variants in TERT and TERC are known to cause d*yskeratosis congenita (DC) and related diseases. These patients have impaired telomere elongation causing stem cells to enter premature replicative senescence and/or apoptosis as telomeres become critically short. This explains the major impact of the disease on highly proliferative tissues such as the bone marrow and skin. However, variants in *TERC* and *TERT* are not always fully penetrant and in some DC families disease causing variants are seen in asymptomatic family members. It is therefore challenging to determine whether a new variant is pathogenic or not. Over the last three years we have identified 19 telomerase variants in patients with DC and related disorders. Analysis of these variants, taking into account familial segregation and functional studies, enabled us to categorise them into three groups; (a) disease causing, (b) bystanders and (c) status uncertain. Remarkably, these investigations identified families with disease causing variants where the mechanism of disease inheritance is novel; one family has triallelic mutations in both telomerase genes and in two families an epigenetic-like mechanism is at play. We have constructed a diagnostic algorithm which may be useful for the categorisation of uncharacterised telomerase variants in the future. This study therefore highlights that telomerase variants have highly variable functional and clinical manifestations and require thorough investigation to assess their pathogenic contribution.

Grants: MRC, Children With Cancer

PS11.059
Phenotypic overlap of dyskeratosis congenita with other syndromes identified through exome sequencing

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The clinical presentation of dyskeratosis congenita (DC) and its allelic variants encompass a wide range of features including abnormal skin pigmentation, nail dystrophy, leukoplakia and bone marrow failure. Pathologically DC is characterized by selective exhaustion of highly proliferative cells that have critically shortened telomeres. To date causal mutations in ten telomere biology genes have been identified.
Through whole exome sequencing on uncharacterized DC patients, we identified biallelic variants in four families in the genes *DNAJC3, GRHL2, LIG4* that have been associated with other diseases (APCHD, Ectodermal dysplasia, Ligase IV syndrome, respectively) which have overlapping clinical phenotypes with DC. This raises the question, if these variants are disease-causing as we believe, then how wide is the phenotype of dyskeratosis congenita?
The table emphasises the large degree of clinical overlap seen in these patients with the diagnostic spectrum of phenotypes seen in DC as well as the diseases associated with the causal variants. These patients were referred with a suspected diagnosis of DC but based on the identified mutation, an alternative diagnosis should be considered. Telomere length tends to be short in most DC patients but is normal in these individuals. The outcome from this exome sequencing project highlights the difficulty in assigning a clinical label to a highly variable disease phenotype. It also suggests potential biological overlap of DC with other syndromes.
Table 1

Grants: MRC, Children with Cancer

Table 1

Phenotypic overlap between DC and other syndromes

	Dyskeratosis Congenita	Ataxia combined cellular and peripheral with hearing loss and diabetes mellitus - ACPHD (OMIM)	Family 1-DC	Ectodermal dysplasia syndrome (OMIM)	Family 2-DC	Family 3-DC	Ligase IV syndrome (OMIM)	Family 4-DC overlap
Mutated gene (variant identified)	various	*DNAJC3*	*DNAJC3* (p.Arg393X, hom)	*GRHL2*	*GRHL2* (p.Pro405Thr, hom)	*GRHL2* (Ile482Lys, hom)	*LIG4*	*LIG4* (p.Arg814X and p.Lys424ArgfsX20)
Nail dystophy	>60% cases			yes	yes	yes		
abnormal skin pigmentation	>60% cases			hyperkeratosis	yes	yes	yes	yes
Bone marrow failure	>60% cases		yes				yes	yes
Leukoplakia	>40% cases		yes	yes	yes	yes		
Developmental delay	>20% cases		yes				yes	
Microcephaly	>20% cases		yes				yes	yes
Growth retardation	>20% cases	yes	yes	yes	yes	yes	yes	yes
Dental problems	>10% cases			yes	yes	yes		
Oesophageal stricture	<10% cases		yes	yes				
Ataxia	<10% cases	yes	yes					
Gonadal abnormalities	<10% cases		delayed puberty		yes			
Immunodeficiency	<10% cases						yes	yes
Ear abnormalities / deafness	<10% cases	yes	yes	yes				
Diabetes	<10% cases	yes	yes					
Kidney abnormailities	<10% cases				kidney agenesis	recurrent infections		
Short telomeres	<1st centile	unknown	normal	unknown	normal	normal	unknown	normal

PM11.060

Dysmorphology Services: a snapshot of current practices and a vision for the future.

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Dysmorphology concerns the recognition and management of rare, multiple anomaly syndromes. Genomic technologies and software for gestalt recognition will re-shape dysmorphology services. In order to reflect on a model of the service in the post-Genomic era, we compared the utility of dysmorphology consultations in two Mediterranean cities, Athens, Greece and Afula, Israel (MDS), the Manchester Centre for Genomic Medicine, a UK service with dysmorphology expertise (UKDS) and the DYSCERNE, digital service (DDS). We show that it is more likely that Chromosome Microarray Analysis will be performed if suggested in the UKDS rather than in the MDS; this most probably reflects the difference of access to genetic testing following funding limitations in the MDS. We also demonstrate that in terms of achieved diagnosis, the first visit to a dysmorphology clinic is more significant than a follow-up. We show that a confirmed syndrome diagnosis significantly decreases the requests for other, non genetic, laboratory investigations. Conversely, it increases the requests for reviews by other specialists and, most significantly (t-test 8.244), it increases further requests for screening for possible associated complications. This is the first demonstration of the demands, on a health service, following the diagnosis of a dysmorphic condition.

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PS11.061

Severe scoliosis in a girl with Temple syndrome due to isolated imprinting defect on human chromosome 14q32.2

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ABSTRACT

Human chromosome 14q32.2 contains an imprinted gene cluster. Maternal uniparental disomy of chromosome 14, paternal deletions and loss of methylation at the intergenic differentially methylated region (IGDMR) result in a distinct human phenotype known as "maternal uniparental disomy 14 phenotype" also referred to as "Temple syndrome". The condition was first described in 1991 and is characterized by pre- and postnatal growth retardation, hypotonia, early-onset puberty, truncal obesity, small hands and feet and feeding difficulty.

To date, only seven patients with a primary epigenetic aberration confined to the 14q32.2 IGDMR have been described. The present report concerns a 13-year-old girl with a maternal uniparental disomy 14 phenotype who displayed an isolated imprinting defect at the maternally expressed gene 3 (*MEG3*) locus and developed rapidly-progressing scoliosis that required surgical treatment. We also review published reports on patients with an epimutation.

PM11.062

Whole-exome sequencing in two sisters with severe hypotonia and hyporeflexia, cognitive deficit, and epilepsy

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We identified two Italian sisters with severe cognitive and psychomotor delay, language defects, epilepsy, nystagmus, generalized hypotonia, heart rhythm abnormalities, osteotendinous hyporeflexia. Exome sequencing of the two affected siblings and their non-consanguineous healthy parents allowed circumscribing five genes with potentially causative variants. These rare and predicted to be deleterious variants were found either as homozygote within the MEOX2 gene or as compound heterozygote in exons of the GNB5, PHLDB3, SLC22A1 and RRN3 genes. The validation of these findings through identification of more patients with mutations in these genes, in vivo complementation, as well as engineering of animal models is warranted.

Among these candidate genes, we are currently focusing on the variant identified in GNB5 gene. The paternal allele carries a missense variant positioned in the last nucleotide of the second exon of this gene. This variant alters splicing accuracy, as determined by RT-PCR followed by direct sequencing of the amplified products. The maternal allele variant is a stop gain that targets transcripts to nonsense-mediated mRNA-decay. GNB5 is the fifth member of the heterotrimeric G-protein β-subunit family, involved in signal transduction receptors and effectors. Mice lacking Gnb5 exhibit markedly abnormal neurological phenotype including impaired development, tiptoe-walking, motor learning and coordination deficiencies, and hyperactivity. Further, it controls the deactivation of retinal phototransduction and the proper functioning of retinal bipolar cells in KO mice. Thus, Gnb5 may regulate dendritic arborization and/or synapse formation. These findings bear a resemblance to some of the reported symptoms in the examined patients.

PS11.063

Clinical utility of exome sequencing as a first-tier molecular test in infants suspected of having a monogenic disorder

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Single-gene disorders are a common cause of morbidity and mortality in infants. Diagnosis can be challenging as the clinical presentation is frequently undifferentiated or incomplete in the early stages. Individual disorders are rare, and often genetically heterogenous. This commonly results in extensive, protracted, costly and invasive diagnostic investigations in this patient group.

We prospectively evaluated exome sequencing as a first-tier molecular test in 37 infants suspected of having a monogenic disorder. All participants were less than 2 years of age, and 40% were less than 3 months of age. Infants with pathogenic CNVs and those who had previously undergone singe gene sequencing were excluded. Exome sequencing with targeted phenotype-driven analysis occurred in parallel with standard investigations, including single gene Sanger sequencing when clinically indicated. The most common indication for testing was the presence of multiple congenital abnormalities and dysmorphic features (54%), followed by suspicion of a neurometabolic disorder (20%). Exome sequencing resulted in a molecular diagnosis of 21 genetic conditions in 20 infants, giving a diagnostic rate of 54%. Four relatives received a genetic diagnosis following cascade testing, and 10 couples were identified as being at high risk (25% or 50%) of recurrence in future pregnancies. By contrast, a molecular genetic diagnosis was reached in 20% using standard approaches in the same patient group.

Employing exome sequencing as a first-line molecular test in selected infants has the potential to considerably shorten the diagnostic process, improve diagnostic yield, guide management, and enable accurate recurrence risk counselling in a timely manner.

PM11.064

Eyebrow abnormalities as a suggestive feature for the diagnosis of multiple congenital anomalies syndromes

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Eyebrow abnormalities are relatively rare birth defects that could be highly suggestive for the diagnosis of multiple congenital anomalies (MCA) syndromes. To confirm this hypothesis, we have selected 84 cases from our own experience with marked eyebrow abnormalities. All patients had a specific diagnosis confirmed with genetic tests in most of the cases. We have identified different types of eyebrows based on specific criteria like facial distribution, sparseness, thickness or uniformity. The most common abnormalities in our group were diamond-shaped eyebrows (32% of cases), synophris (18% of cases) and sparse eyebrows (17%), whereas the least common

ones were linear and lowset, as well as thick eyebrows (4% each) and bushy eyebrows (1% of cases). Each eyebrow pattern has been associated with specific syndromes and based on our own experience and literature data we have identified for every pattern the most commonly associated clinical features, making the association highly suggestive for the diagnosis. Clinical data will be illustrated with pictures.
In conclusion, we appreciate that Clinical Genetics still has a place within the Genomic Era, the recognition of suggestive clinical features being very useful for MCA diagnosis in developing countries, where financial resources are limited and detailed molecular testing is not always available in daily practice. This leads to a more specific diagnosis and increases the cost-effectiveness of genetic testing.

PS11.065
Novel mutations in PIEZO1 cause an autosomal recessive form of Generalised Lymphatic Dysplasia

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We have identified *PIEZO1* mutations that cause a form of autosomal recessive GLD which is characterised by a high incidence of non-immune hydrops fetalis and chronic peripheral primary lymphedema. This may be lethal in utero or in the neonatal period. However, it may fully resolve and present later in childhood with four limb lymphoedema with facial oedema and a susceptibility to facial cellulitis with intermittent swelling. Some of the patients have intestinal lymphangiectasia or pleural effusions.
Whole exome sequencing was performed in three families and novel variants in only one gene, *PIEZO1*, were identified under the criteria of an autosomal recessive disease. The whole coding region of the gene was Sanger sequenced in 10 unrelated individuals of similar phenotype. In total, we have identified nine homozygous or compound heterozygous mutations in six unrelated GLD families; three nonsense, four missense, and two splice site mutations. The missense mutations were located in evolutionary conserved residues and predicted to have damaging effects on the protein. The splice variants caused exon skipping and the nonsense mutations affected PIEZO1 expression. RT-PCR and western blot were used to investigate the effect of the identified variants on gene and protein expression. A skin biopsy was investigated and lack of initial lymphatics was found.
Mutations in PIEZO1 have previously been reported to cause autosomal dominant DHS but our report suggests that biallelic mutations in this gene cause GLD. Therefore, it suggests that PIEZO1 has a role in the development of lymphatic structures. This work was supported by the BHF, the Newlife Foundation for Disabled Children and the NIH.

PM11.066
Identification of the first gene involved in Goldenhar syndrome

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The oculoauriculovertebral spectrum (OAVS, OMIM 164210) or Goldenhar syndrome is a developmental anomaly involving the first and second branchial arches. This pathology represents one of the most common congenital craniofacial disorder, with a prevalence around 1/26,000 births. Main features include facial asymetry resulting from maxillary and/or mandibular hypoplasia, hemifacial microsomia, unilateral or bilateral ear anomalies, ocular defects, and vertebral malformations. To date, although various chromosome abnormalities have been associated with OAVS and several candidate genes have been screened, the genetic etiology of this pathology remains largely unknown.
For the present study, a project with national recruitment established a cohort of 156 patients with OAV spectrum. In order to determine the molecular basis of Goldenhar syndrome, we performed exome sequencing in trios including selected patients with Goldenhar syndrome and their healthy parents. We identified a nonsense mutation in GOLD1 gene, a poorly characterized transcription factor known to be involved in ear development. Screening of the cohort identified a missense mutation in a second patient. Interestingly, GOLD1 belongs to a genetic environment within a paralogon that was already involved in another Copy Number Variant associated with OAVS. Functional studies by transient knockdown in zebrafish model evidenced gold1 as craniofacial cartilages architecture key factor.
In conclusion, we report GOLD1 as the first gene involved in Goldenhar syndrome. Future identification of others genes should allow a better understanding of molecular processes leading to OAVS.

PS11.067
Three siblings with non-immune hydrops fetalis with gracile bones and dysmorphic features: second report in the literature

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A brother and a sister from a consanguineous family was first described by Abbot et al. in 2008 (MIM 613124). They both had non-immune hydrops fetalis with gracile bones and dysmorphic features. They died after an hour from birth due to respiratory insufficiency.
We here report three female siblings from a consanguineous Turkish family, who had non-immune hydrops fetalis, dysmorphic facial appearance, and bone fractures. They were born at 36, 33 and 33+6 week gestation by caesarean section due to malpresentation, respectively. All of siblings had similar facial dysmorphism with down-slanting palpebral fissures, low-set ears, short and upturned nose, long and smooth philtrum, narrow and high palate, and short neck. Radiographic studies of the first child revealed fractures of bilateral humerus and right femur, second child had fractures of left humerus and left femur, whereas last child had only left humerus fractures. The siblings died after three days, forty-three days and nine days from birth, due to respiratory problems, respectively. We suggest that our patients are similar with those of Abbott et al. indicating a new autosomal recessive condition. To elucidate this phenotype, molecular studies are still in progress.

PM11.068
Multiple Congenital Anomalies in two Boys with Mutation in HCFC1 and Cobalamin Disorder

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The cobalamin type C deficiency is a rare condition that results from impaired biosynthesis of both methylcobalamin (MeCbl) and adenosylcobalamin (AdoCbl). Hemizygous mutations of the HCFC1 gene explain the majority of clinically and biologically compatible cblC patients without MMACHC mutations (OMIM 309541). We report a family with two maternal half-brothers with multiple congenital anomalies and HCFC1 gene mutation in the second Kelch domain. Both presented with dysmorphic features (flat profile, cleft lip for one), increased nuchal translucency, prenatal onset microcephaly and hypospadias. Additionally to early onset intractable epilepsy and profound neurocognitive impairment, this familial observation suggests that HCFC1 gene should be considered in boys with midline malformations, even without proven cobalamin C deficiency.

PS11.069
Diagnostic dilemma - Hidrolethalus syndrome

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Hydrolethalus syndrome is a rare clinical entity including as main characteristics hydrocephalus, absent midline structures, polydactyly and lethality. The vast majority of the cases have been reported in Finland. We present two Romanian newborns with severe malformations. The first case is a male fetus. The fetus was delivered by caesarian section at 28 weeks of gestation, with hydrocephaly, microphtalmia, cheiloschisis, micrognathia, low set ears, bilateral postaxial poydactyly with six fingers of the hand, preaxial and postaxial polydactyly of the feet with seven toes and syndactyly of 5th and 6th. The fetus lived 20 minutes. Autopsy showed also laryngeal stenosis, palatoschisis, lissencephaly, agenesis of corpus callosum, classic open book appearance of the brain. The second case is a feminine fetus, delivered by caesarian section at 29 weeks of gestation. She lived the 10 minutes. She

showed severe dysmorphia with true anophthalmia, arhinia, median cleft upper jaw, very small lower jaw and small ears. She had right hand with polydactyly and bilateral syndactyly of 2th and 5th toes. Autopsy showed cerebral hemispheres open-book and the occipital keyhole images, agenesis of corpus callosum and lissencephaly. The right lung had two lobes, the left lung one lobe. Sequencing analysis of HYLS1 and KIF7 genes did not identify any pathogenic variants. The dilemma is the discordance between the presence of majority of the specific signs of the syndrome and absence the mutations in the so far causative genes. These cases with overlapping manifestations of the hydrolethalus syndrome might be in fact another hydrolethalus syndrome or the syndrome is etiologically heterogeneous.

PM11.070

A new homozygous *IGF1R* mutation defines an autosomal recessive form of SHORT syndrome characterized by developmental delay and marked progeroid appearance.

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Insulin-like growth factors I (IGF-I) is essential for pre and post-natal somatic growth and cellular proliferation. The binding of IGF-I to IGF1R leads to receptor autophosphorylation resulting in recruitment of cytoplasmic components of multiple downstream signalling pathways, including the PI3K/Akt and MAPK/Erk pathways, which are important for cell survival and cell growth.
Here, using exome sequencing, we identified and characterized a new homozygous c.2201G>T mutation in the IGF1R gene in a female patient, born from consanguineous parents, showing very high IGF-I levels and clinical features of SHORT syndrome plus developmental delay, CNS defects, and marked progeroid appearance. This mutation cosegregated in the heterozygous state in the parents and other relatives, which show a milder phenotype.
Based on a bioinformatics analysis, the c.2201G>T mutation was predicted to cause an alternative splicing event that we confirmed by RT-PCR followed by direct sequencing of amplified products. This event causes an aberrant isoform that adds 25 aminoacids after the proline at codon 733, predicted to be damaging and causing dramatic structural changes of the protein.
Functional studies using primary dermal fibroblast cultures obtained from affected and healthy individuals revealed a lower IGF1R protein expression in both homozygous and heterozygous fibroblasts. Furthermore, decreased IGF-1R content affects IGF-I signalling, as showed by a reduction of IGF-I-dependent Akt phosphorylation and IGF-IR auto-phosphorylation.
This study provides valuable insights into the pathophysiological and phenotipical consequences caused by loss of IGF1R function.

PS11.071

A Germline mTOR Mutation in Aboriginal Australian Siblings with Intellectual Disability, Dysmorphism, Macrocephaly and Small Thoraces.

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The Australian Desert, a new familial phenotype and a global search for functional studies to support potential drug repurposing. We report 3 Aboriginal Australian siblings with a unique phenotype which overlaps with known megalencephaly syndromes and RASopathies, including Costello syndrome. A gain of function mutation in mTOR was identified and represents the first reported human condition due to a germline, familial mTOR mutation. We describe the findings in this family to highlight i) that the path to determination of pathogenicity was confounded by the lack of genomic reference data for Australian Aboriginals and that ii) the disease biology, functional analyses in this family and studies on the Tuberous Sclerosis Complex support consideration of an mTOR inhibitor as a therapeutic agent

PM11.072

Oligonucleotide array CGH analysis of a cohort of 220 patients with intellectual diability, developmental delay and multiple congenital anomalies

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Array CGH (aCGH) enables genome wide investigation of copy number changes at high resolution and have recently been implemented as a clinical diagnostic tool. In this study, a high resolution oligonucleotide chromosomal microarray was applied in an Iranian cohort of 220 patients with DD/ID and multiple congenital anomalies: male;114, female:116, aged 7days- 20 years. DNA extracted from peripheral blood cells were analyzed with CytoChip_ISCA_4x180 oligo array. The imbalances were confirmed by FISH or MLPA. We considered copy number variants (CNVs) as pathogenic if the variant was responsible for a known syndrome, encompassed gene/s of known function, occurred de novo or, if inherited, the parents was variably affected and/or the involved gene/s had been reported with DD/ID and multiple congenital anomalies. aCGH identified 39 clinically significant microdeletions and 18 microduplications in 23.6% (52/220) patients, with size of aberrant regions ranging from 39kb to 24.2Mb. 26.3% (15/57) of the copy number variant (CNV) detected corresponded to well-known microdeletion and microduplication syndrome (Williams, Digeorge, prder-Willi, kabuki, WolfHirschhorn, Tar, Reiger, Potoki-lupski and Mitochondrial complex deficiency IV syndrome). 61% (35/57) of the detected CNVs were de novo, 26% (15/57) inherited and 12% (7/57) unknown. We also identified two novel CNVs, one at 9p11.2-p13.1 and the other at 10p12.1 as a pathogenic submicroscopic CNV. Our study provides further evidence of the high diagnostic yield of aCGH for genetic testing of patients with DD/ID and unexplained multiple congenital anomalies.

PS11.073

2q22.1q22.3 deletion, upstream ZEB2, causes intellectual disability, myoclonic epilepsy, omphalocele, genital anomalies and facial similarities with Mowat-Wilson syndrome

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BACKGROUND
Array-CGH has improved the diagnostic yield in patients with intellectual disability (ID) and/or multiple congenital anomalies (MCA), allowing the definition of novel microdeletion and microduplication syndromes.
CASE REPORT We report a boy with severe ID, dysmorphisms, myoclonic epilepsy, thin corpus callosum, omphalocele and chordee. He presents distinctive facial features comprising large, prominent forehead, thick eyebrows, deep-set eyes, strabismus, wide nasal bridge, bilateral epicanthus, large ear lobes, prominent, rounded nasal tip, deep philtrum, open, large mouth with M-shaped upper lip, small teeth and pointed chin, which were somewhat evocative of Mowat-Wilson syndrome. He has wide-spaced nipples and prominent finger pads in hands and feet.
RESULTS
Array-CGH identified a de novo 2q22.1q22.3 (140,509,156-144,980,623) deletion, comprising the LRP1B, KYNU, ARHGAP15 genes and partially the GTDC1 gene. Interestingly, the ZEB2 gene, responsible for Mowat-Wilson syndrome, is located about 150Kb downstream the deletion.
DISCUSSION
Two other patients have been previously reported with de novo similar deletion and no other genomic rearrangement:
- in DECIPHER, patient 1607 (del 139,813,180-145,063,389), who presents ID, craniofacial dysmorphism, strabismus, absent nipples, and prominent fingertip pads
- in the literature, Mulatinho et al describe a Brazilian patient (del 138,750.000-144.750.000) presenting ID, lack of speech, craniofacial dysmorphism, omphalocele, hypospadias and cryptorchidism.
CONCLUSION
A novel case of 2q22.1q22.3 deletion is documented, defining a rare, but recognizable, ID/MCA syndrome, bringing the total number of known cases to 3. Omphalocele and genital anomalies are present in 2 of the 3 patients. Patients present similar craniofacial features, somewhat evocative of Mowat-Wilson syndrome, suggesting a possible positional effect over ZEB2.

PM11.074

Effectiveness and costs of whole exome sequencing compared to traditional diagnostic investigations in children with complex neurological disorders

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Introduction: A systematic evaluation of whole exome sequencing (WES) diagnostic yield and assessment of the costs associated with implementing WES in the clinic is currently lacking. We determine the increased diagnostic yield and provide a thorough cost analysis of the clinical care prospectively with WES compared retrospectively to care without WES.

Materials and Methods: Seventeen children of healthy, unrelated parents were randomly selected from the 2011 patient population at the Sylvia Toth Center (STC; Utrecht, the Netherlands), a specialized center for children with complex neurological disorders and diverse phenotypes. WES was performed on Illumina HiSeq 2500. In parallel, the clinical records of patients were obtained and a comprehensive cost summary of medical treatments, hospital visits, and all other resource use was compiled per patient. This cost was then compared to the cost of care using WES, assessed prospectively.

Results: The diagnostic yield in this patient cohort is 18%, confirming past studies' diagnostic yields on intellectual disability cohorts. The three mutations detected are in genes recently associated with intellectual disability (ANKRD11, CTNNB1, ANDP), and the mutations are all heterozygous de novo frame-shift deletions resulting in protein truncation. In addition, strong candidate genes (HNRNPU, CHD4) have been identified.

Conclusions: The increased diagnostic yield and fractional increase in total overall price in the cost of care for these patients suggests that WES should be implemented early in patient care for similar centers that have patient populations for whom providing a diagnosis is both difficult and expensive.

PS11.075

Interstitial deletion 6p21.2-p21.1: characterization by array-CGH and phenotype description

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Deletions in the short arm of chromosome 6 represent a rare genetic alteration and small number of cases was described, frequently about telomeric region. The number of patients identified with microdeletion syndrome has been increasing significantly with widespread application of high-resolution genome analysis technologies such as array comparative genomic hybridization (array-CGH).

We identify a 6,295Mb interstitial deletion at 6p21.2-p21.1 by array-CGH. This region encompasses fifty-seven genes cataloged at OMIM.

The patient was a seven years old girl with mental retardation, neurosensory deafness, facial dymorphism, obesity beginning in infancy and atopic dermatitis.

The comparison genotype/phenotype has been done and six genes whose function is known and correlated have been identified. The consequences of haploinsufficiency of GLP1R, MOCS1, TREM2, GNMT, PEX6 and CUL7 in this case have been debated.

Individually, none of the identified genes encompasses all findings in patient, however could be contributing in the phenotype.

PM11.076

Two cases with a rare chromosomal abnormality: isochromosome 18p

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Tetrasomy 18p, one of the most commonly observed isochromosomes, consists of two copies of the p arms on chromosome 18[i(18p)]. It has a prevalence of 1/140,000-180,000 live births and affects both genders equally. Tetrasomy 18p syndrome is characterized by nonspecific morfologic features; low birth weight, microcephaly, low-set ears, strabismus, abnormalities in muscle tone and deep tendon reflex. Feeding difficulties and developmental delays are also followed.

We present two cases with *de novo* tetrasomy 18p. In the first case we report a *de novo* tetrasomy 18p in a female dysmorphic child with delayed psychomotor development and hypotonia. The physical examination showed: microcephaly, strabismus, low-set ears, bilateral epicanthic folds, broad nasal bridge and tented mouth with slightly everted upper lip. The small metacentric marker chromosome was identified by conventional cytogenetic analysis and fluorescence *in situ* hybridization (FISH) as i(18p) in the proband with the karyotype 47,XX,+i(18)(p10). In the second case we present a prenatally detected mosaic isochromosome 18p with the karyotype 47,XY,+i(18)(p10)[30]/46,XY[29] by amniocentesis. Interphase FISH analysis of stimulated cultured cord blood lymphocytes showed 2% (6/306) mosaicism for tetrasomy 18p. The pregnancy was terminated.

Array comparative genomic hybridization (aCGH) confirmed the diagnosis of tetrasomy 18p molecularly and revealed a 14 Mb triplication at 18p11.32p11.21 in the first case. In the second case a 14 Mb duplication at 18p11.32p11.21 was characterized by aCGH. The parental origin of the isochromosome was determined based on a variety of short tandem repeats and was found to be maternal in both cases.

PS11.077

Growth and growth hormone in 15 children with Kabuki syndrome

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Introduction

Kabuki Syndrome (KS; OMIM 147920) is a congenital anomaly/intellectual disability syndrome caused by a mutation in the KMT2D or KDM6A gene. Children with KS have a spectrum of clinical features, one of the key features is postnatal growth deficiency.

Mutations in KMT2D gene, encoding an H3K4 histone methyl transferase, act as an epigenetic transcriptional activator during growth and development. As for the KDM6A mutation, no molecular cause for growth retardation has been described.

GH deficiency has been reported in KS but in the majority of children with KS no cause for small stature has been found. We studied the growth hormone and IGF-I pattern in order to learn more about a possible mechanism involved in this postnatal growth retardation.

Methods

Currently, we have assessed 15 KS children (age 3-10 years old, 6.44±2.29) with a known KMT2D or KDM6A mutation. Height was variable, with a mean height SDS of -2.38 ±1.41. Both clonidine (CLO) and arginine (ARG) were used for stimulation. GH and IGF-1 were measured according to international standard.

Results and Conclusion

Growth hormone deficiency was present in 4 of 15 (26.67%) children. Apparently growth hormone deficiency is not the only cause for small height in KS patients. In addition, in 2 children the GH tests showed a tendency to GH resistance, although no one actually met the defined criteria. Further research is necessary to determine the underlying cause of growth retardation in the majority of KS patients.

Currently we are performing a clinical study with growth hormone in children with Kabuki syndrome; the results are pending but promising.

PM11.078

Kapur-Toriello Syndrome: a further case report and expansion of the phenotype

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Kapur-Toriello syndrome (KTS) is a rare multiple congenital abnormalities syndrome first described by Kapur and Toriello in 1991. We present a new case, taking the total number reported in the literature to six.

With gradual expansion of the cohort, evidence is emerging for several key features including bilateral cleft lip / palate, a distinctive nose, low set ears, ocular, cardiac, intestinal and brain malformations. Other features seen less commonly in previous cases, but which our case adds evidence for, include growth retardation, digital abnormalities, short stature and a short, wide neck.

Structural pituitary abnormalities associated with growth hormone deficiency have not been previously reported in patients with KTS, although short stature and growth retardation have been frequently noted. To our knowledge, our patient is the first with KTS to receive growth hormone therapy. It would therefore seem appropriate to monitor growth hormone levels in patients with KTS presenting with short stature.

Autosomal recessive inheritance has previously been suggested. As this is the fourth case with a single affected individual born to non-consanguineous parents, it raises the possibility of autosomal dominant inheritance.

With so few documented cases of KTS, it is important for new cases to be

reported so that the clinical phenotype may be better delineated. As whole genome sequencing becomes increasingly available, we hope that a molecular cause for this rare syndrome may soon be identified.

PS11.079

KBG syndrome: a series of 20 French patients

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Mutations and deletions of the *ANKRD11* gene are known to cause KBG syndrome. We report a series of 20 French patients with KBG syndrome including 3 families. Molecular anomalies consisted of 9 mutations and 8 deletions involving at least part of the *ANKRD11* gene. The 9 mutations were identified by targeted sequencing of the gene, following clinical diagnosis of KBG syndrome. The age at diagnosis ranged from birth to 65 years. All mutations were loss of function and were clustered in the largest exon. The size of deletions ranged from 127kb to 1.2Mb. There were no significant clinical differences between mutated and deleted cases. Facial gestalt was characteristic in all cases, and allowed the diagnosis even in young children. Deafness was present in 25%, short stature in 55%. Interestingly, the teeth were considered as normal in 3 adult patients and two patients exhibited a caudal appendage. In most patients, intellectual level ranged from borderline intelligence to mild/moderate intellectual deficiency. A single patient had severe ID. Two had epilepsy and behavioral problems were noted in 20%. From an epidemiological point of view, our study confirms the high rate of familial cases. All cases with a mutation were recruited from two French centers, whereas the 8 16q24.3 deletions identified by array-CGH deletions were recruited through a national network. We postulate that mutations are probably more frequent than deletions but remain undiagnosed. Diagnosis is clinical on the basis of typical facial features and similarities with Cornelia de Lange syndrome may be striking.

PM11.080

A review of the phenotype in Microcephaly with or without Chorioretinopathy, Lymphoedema or Mental Retardation (MCLMR) associated with KIF11 mutations

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Microcephaly with or without chorioretinopathy, lymphoedema, or mental retardation (MCLMR) (MIM #152950) is a rare autosomal dominant condition. The causative gene, kinesin family member 11 (KIF11) was identified in 2012. KIF11 mutations were found in individuals with and without lymphoedema. To further delineate this condition, we collected the clinical details of thirty seven individuals from twenty two families, all with KIF11 mutations.

The condition arose de novo in 40% of cases where parental results were available. In our cohort, 86% had microcephaly, 78% had an ocular abnormality consistent with the diagnosis, 46% had lymphoedema, 73% had mild-moderate learning difficulties, 8% had epilepsy and 8% had a cardiac anomaly. We identified three individuals with KIF11 mutations, but no clinical features of MCLMR demonstrating reduced penetrance. The variable expression of the phenotype and presence of mildly affected individuals indicates that the prevalence may be higher than expected, and we would therefore recommend a low threshold for genetic testing.

Our data suggests that mutations in KIF11 cause a variable spectrum of clinical features. Not only is the microcephaly variable, the ocular abnormalities are also variable with chorioretinopathy being the most prevalent. Recently a series of cases with retinal detachment mimicking FEVR have been reported suggesting a broader spectrum of ocular manifestation than originally suggested. Our review will explore and summarise the relationship between the KIF11 genotype and some of the major, phenotypic characteristics of MCLMR identified to date. This work was funded by BHF.

PS11.081

New insights and broader spectrum of lissencephaly including a definition of a novel subtype.

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Introduction: Lissencephaly (LIS), the most severe malformation of neuronal migration, is characterized by broad or absent gyri and abnormally thick (12-20 mm) cortex. Several patterns and syndromes are known including the less severe variant subcortical band heterotopia (SBH), but rapidly increasing knowledge requires revision of the current classification.

Materials and Methods: We reviewed brain scans, clinical and molecular findings for 129 LIS patients ascertained from 2009-2014 and grouped them by severity (LIS grade 1-6), gradient (diffuse vs. anterior or posterior), and presence of other malformations.

Results: Posterior predominant LIS grade 3 (27%) followed by diffuse LIS or SBH (17%) due to mutations of LIS1 and DCX were the most common forms. All other LIS patterns were less frequent (2-9%) with anterior predominant forms significantly less frequent than posterior predominant forms. Recognizable tubulinopathies accounted for 10% of patients (grade 4 LIS with atypical mixed pachygyria-microgyria). This is the only group lacking the consistent anterior or posterior gradient characteristic of LIS. In addition, we recognized a novel anterior predominant "thin" LIS (cortex 5-7 mm; normal is 3-4 mm) with reduced number of gyri and shallow sulci. When combined with severe cerebellar hypoplasia this variant is caused by mutations in RELN or VLDLR. However, 8 patients had "thin" LIS with a normal cerebellum. None had mutations in the known LIS genes. Based on high percentage of homozygosity in SNP arrays in several of these patients, we strongly suspect autosomal recessive inheritance. We will present more detailed diagnostic criteria and updated results of molecular testing.

PM11.082

Diagnostic yield of targeted next generation sequencing panel for cortical malformations

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Malformations of the brain cortex development (MCD) are rare diseases diagnosed by MRI scan and are classified into proliferation disorders(microcephalies and megalencephalies), disorders of neuronal migration (lissencephaly, cobblestone malformation and heterotopia) and disorders of cortical organization (polymicrogyria and schizencephaly). Identification of many genes has improved etiological diagnosis, but genetic heterogeneity makes testing of individual genes unpractical. In Rotterdam a diagnostic custom-made panel for high throughput sequencing of 103 genes involved in MCD, belonging to all three ategories has been developed. About 168 patients have been tested in two years. Most of the patients had no mutation in one or more candidate genes (e.g. *LIS1, DCX, FLNA, ARX, GPR56*), previously tested by Sanger sequencing. The diagnostic yield of the panle

was about 6%. Most diagnoses have been achieved in the group of primary microcephalies, and the least in the group of polymicrogyria, although the latter was the most common indication for the test. Expert post-test clinical and radiological re-phenotyping has led to reclassification of the disease, broadening the phenotypic spectrum of known disorders, as for mutations in the small nuclear RNA coding *RNU4atac*, for *ACTB*, *KIAA1279*, *CENPJ and RELN*. Diagnoses also included cases with insufficient radiological data for appropriate classification. Low-abundance mosaic mutations, e.g. *TUBB2B* and *PIK3R2* that we observed, remain a challenge to detect. When applied as first-tier test, targeted NGS panels can be cost-effective for the diagnosis of MCD, without the drawbacks of unsolicited findings. However these preliminary data underpin the etiological heterogeneity of MCD and the need for collaborative research efforts.

PS11.083

Skeletal dysplasia may be a feature of Meckel syndrome type 9 (MKS9), a rare entity caused by *B9D1* mutations

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INTRODUCTION: Meckel Syndrome (MKS) is a genetically heterogeneous recessive ciliopathy presenting in fetal life with multiple malformations, of which occipital encephalocele is the most characteristic. MKS type 9, caused by *B9D1* mutations, is a rare entity with only two published cases so far, and none of them had a skeletal phenotype.
MATERIALS AND METHODS: An unrelated couple lost four fetuses due to malformations consistent with MKS. Fetal DNA was collected from CVS or spleen and subjected to 6.0 SNP-array-based copy number analysis, haplotype analysis, and finally whole-exome sequencing (WES) analysis of a ciliopathy-related gene panel. Whole body X-ray was performed on fetus 4.
RESULTS: 6.0 SNP-array and Sanger sequencing of the *MKS1*-gene were normal. Fetus 1 and 2 shared haplotypes around *NPHP3, TMEM67, B9D1,* and *B9D2.* Only in *B9D1* compound heterozygosity for pathogenic variants were detected by the ciliopathy panel and later verified by Sanger sequencing. Ultrasound and X-ray examination of fetus 4 displayed angulated tibiae and radii and a large calvarium.
CONCLUSION: Only four patients with a ciliopathy due to *B9D1* mutations have so far been described, two with MKS and two with Joubert syndrome (JS). Here we expand the severe end of the phenotypic spectrum of *B9D1* mutations, adding skeletal dysplasia to the list of possible manifestations.

PM11.084

X-linked microcephalic primordial dwarfism syndrome in two siblings

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A boy was born at term from non-consanguineous parents with a birth weight of 2130 gr (-3 SD), length of 45 cm (-2.5 SD) and OFC of 30.5 cm (-4 SD). Pregnancy was complicated by oligohydramnios and IUGR. Facial dysmorphism was noted at birth including blepharophimosis, stubby nose and thick columella, mild retrognathia, and posterior cleft palate. Cerebral imaging showed bilateral cysts of the choroid plexus and mild enlargement of the cerebral ventricle. Auditory evoked potentials suggested sensorineural deafness. He had subsequent developmental delay and growth retardation. Hypsarythmia occurred at age 9 months. He died from status epilepticus. His young brother, born at term with a birth weight of 3090 gr (-0.8 SD) and OFC of 31.5 cm (-3.5 SD) had similar dysmorphism, including blepharophimosis and cleft soft palate. Subsequently, he presented severe developmental delay, feeding difficulties and gastro-esophageal reflux. Seizures appeared at the age of 1 year. Aged 13.8 year, his weight is 15.0 kg (-4 SD), height 111 cm (-6 SD) and OFC 44.5 cm (-7 SD). He is unable to walk or speak. Clinically, these patients, originally reported as blepharophimosis-MR syndromes, share similarities with Juberg-Marsidi syndrome. Array CGH was normal. Whole exome sequencing identified a hemizygous mutation in a gene located in Xp11.22, affecting a highly conserved amino-acid, and predicted, in silico, to be pathogenic. This mutation was present in both siblings, the mother and maternal grand-mother but absent in another male maternal relative. X chromosome inactivation and functional studies are currently underway.
This work was supported by a FIRS grant from the CHU Liège, Belgium.

PS11.085

A review of the spectrum of KIF11 mutations causing Microcephaly with or without chorioretinopathy, lymphoedema, or mental retardation (MCLMR)

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Mutations in *KIF11* cause microcephaly with or without chorioretinopathy, lymphoedema, or mental retardation (MCLMR, MIM #152950) which is a rare autosomal dominant condition. MCLMR can be de novo or inherited. The phenotype is variable, often displaying mild-to-severe microcephaly plus any of the following: ocular abnormalities, lymphoedema, and learning difficulties. This report further expands the molecular spectrum of *KIF11* variations.
Previous investigations have identified and reported a total of 50 cases. We have screened another cohort of 28 unreported, unrelated MCLMR subjects for mutations in *KIF11* by Sanger sequencing. We found 15 mutations in 17 subjects; one variant had previously been reported. A variety of mutations were identified including a synonymous mutation (c.2922G>A) in two unrelated subjects, which we show has an impact on splicing. This is the first report of a synonymous mutation in *KIF11* being responsible for causing disease.
Identifying further mutations in this gene supports the role of *KIF11* as contributory to MCLMR. A review of the spectrum of mutations in the *KIF11* mutation positive subjects in this cohort and all published cases (i.e. a total of 67 cases under review) shows that nonsense mutations were most common (33%), followed by frameshift mutations (27%), missense and splice-site mutations (20% each). The mutations are distributed evenly throughout the *KIF11* gene. No mutations were identified in 11 unrelated individuals with clinical features consistent with MCLMR, suggesting genetic heterogeneity associated with this condition. This work was funded by BHF and Newlife Foundation for Disabled Children.

PM11.086

Clinical phenotype of 5q31.1 - q31.2 microdeletion

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Interstitial deletions of chromosome band 5q31 are rather uncommon, only several patients have been reported to date. We report on a case of a 12-year-old girl patient with a de novo 4.19Mb interstitial deletion of chromosome bands 5q31.1-q31.2 detected by whole-genome oligonucleotide microarray analysis. Clinical manifestations in this patient included: failure to thrive, psychomotor retardation, short stature, muscular hypotonia, skeletal anomalies, microcephaly, cleft palate, hand and foot anomalies and dysmorphic features.
Overlapping microdeletions have been previously reported [Rosenfeld et al., 2011: DECIPHER 249028; Kleffman et al. 2012].In these patients microdeletions were rather larger (5.0 - 8.1Mb) and extend distally into 5q31.3. Carriers of larger microdeletions which include subbands 5q31.1 and/or 5q31.3 seem to be more severely affected with congenital malformations, growth anomalies, and severe encephalopathies. No common occurrence of the smallest region of overlap has been observed. Compared to the previously reported patients, severe feeding difficulties, weakness and fatigue of calf muscle and unusual tiredness are unique features only to our patient, which might be due to different sizes and positions of the individual deletions.
In conclusion, this case increases our knowledge of phenotypic consequences of interstitial 5q31 deletions. There has been considerable interest in mapping the smallest region of overlap for this syndrome in order to identify the critical pathogenic genes and establish genotype-phenotype correlations.
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PS11.087

Heterozygous ACTG2 mutations impair binding to actin filaments and cause megacystis microcolon intestinal hypoperistalsis syndrome

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Introduction: Megacystis microcolon intestinal hypoperistalsis syndrome (MMIHS) is a rare congenital disease characterised by severe dysfunctions of the bladder and the intestine. Recently, mutations in the enteric smooth muscle actin γ-2 (*ACTG2*) have been associated with MMIHS. ACTG2, one of the six actin isoforms, is specifically expressed in smooth muscle cells of the enteric organs.
Materials and Methods: In order to confirm the involvement of *ACTG2* in MMIHS development, we sanger sequenced a cohort of sporadic MMIHS patients to determine mutation frequency, and further investigated the molecular pathogenesis associated with *ACTG2* mutations by performing a series of *in vitro* assays and molecular modelling analyses. We also investigated whether these mutations lead to histopathologic abnormalities, and for that we performed immunohistochemistry on intestinal tissue from patients and controls.
Results: Our results showed that heterozygous *ACTG2* mutations were present in all MMIHS patients included in this study. We also determined that the identified mutations inhibited ACTG2 incorporation into the actin filaments, and contributed to reduce contractility. Molecular modelling of ACTG2 mutants confirmed our results, and showed significant changes in the protein structure that likely affect polymerization of ACTG2. Finally, by immunohistochemistry we detected ACTG2 expression in all intestinal layers formed by smooth muscle cells in different stages of human embryonic development. Interestingly, no histopathologic abnormalities were found in patients.
Conclusions: Taken together, our data confirmed that *ACTG2* mutations cause MMIHS and showed that reduced incorporation of ACTG2 into actin filaments affect contractility, and therefore underlies the pathogenesis of MMIHS.

PM11.088
10q26.1 Microdeletion: a New Case Redefines the Critical Regions for Microcephaly and Genital Anomalies

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Distal 10q deletion syndrome is a well characterized chromosomal disorder consisting of neurodevelopmental impairment, facial dysmorphism, cardiac malformations, genital and urinary tract defects as well as digital anomalies. Patients with interstitial deletions involving band 10q26.1 present a phenotype similar to the ones with the distal 10q deletion syndrome, which led to the definition of a causal 600 kb smallest region of overlap (SRO).
In this report, we describe a male patient with an interstitial 4.5 Mb deletion involving exclusively the 10q26.1 segment. He had growth and psychomotor retardation, microcephaly, flat feet, micropenis, and cryptorchidism. The patient's deleted region does not overlap the 10q SRO. We reviewed the clinical phenotype of patients with similar deletions and suggest the presence of two new SROs, one associated with microcephaly, growth and psychomotor retardation, and the other associated to genital anomalies. Interestingly, we narrowed those regions to segments encompassing five and two genes respectively. FGFR2, NSMCE4A, and ATE1 were suggested as candidates for facial dysmorphism, growth cessation, and heart defects respectively. WDR11 was linked to idiopathic hypogonadotropic hypogonadism and Kallmann syndrome. Its haploinsufficiency could play a crucial role in the genital anomalies of these patients.

PS11.089
The first female patient with Myhre syndrome and rare R496C mutation in *SMAD4* gene.

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Myhre syndrome (MYHRS, OMIM 139210) is an autosomal dominant disorder characterized by developmental and growth delay, athletic muscular built, cognitive deficits, skeletal anomalies, stiffness of joints, distinctive facial dysmorphism and deafness. Recently, *SMAD4* (OMIM 600993) was identified by exome sequencing as the disease gene mutated in MYHRS. Previously only three missense mutations affecting Ile[500] (p.Ile500Thr, p.Ile500Val, and p.Ile500Met) have been described in several unrelated subjects with MYHRS. Here we report on a 10-year-old girl with a heterozygous *SMAD4* missense mutation affecting residue Arg[496]. So far this mutation have been described in only three male patients. Our patient presented with striking dysmorphic features and precocious puberty with normal hormonal studies. Her radiological survey shows typical abnormalities including: narrow pelvic, thickened calvarium, flattened vertebrae with enlarged pedicles. But apart from this she had normal psychomotor development and her intelligence is within normal limits. She attends regular school without any learning problems. She has normal soft skin, no stiffness of joints and no muscle hypertrophy. She also has no heart defect, no arterial hypertension nor deafness. She also has no growth delay. Our findings provide further information about the *SMAD4* mutation spectrum in MYHRS and we present the clinical case with rather milder phenotype in comparison to patients with mutations affecting Ile[500].

PM11.090
Exome sequencing resolves a case of myoclonic epilepsy and Fanconi syndrome

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Progressive myoclonic epilepsy encompasses a syndrome of myoclonic seizures and progressive neurological decline caused by a group of rare genetic disorders including lysosomal storage and mitochondrial diseases. The associated aetiological heterogeneity of progressive myoclonic epilepsy poses a diagnostic challenge, yet the application of high throughput sequencing technologies has allowed the identification of rare causal variants in Mendelian diseases such as these. Sodium valproate is a common anticonvulsant drug that is used therapeutically in epileptic disorders, but has been associated with Fanconi syndrome, particularly in severely disabled individuals who are tube fed. We discuss the case of an 8 year old Pakistani boy, with a complex phenotype of myoclonic epilepsy with severe myopathy, severe developmental disability, Fanconi syndrome, pyrexia of unknown origin and a family history of Leigh syndrome. An initial clinical diagnosis of a mitochondrial disorder was made and supported by a marginally decreased level of complex V within the electron transport chain on muscle biopsy. Mitochondrial DNA tests revealed no mutations in m3243A>G or m8344A>G, yet mitochondrial DNA depletion showed an intermediate result of 44% of mean normal levels. Genomic DNA was extracted and sent off for whole exome sequencing and was analysed using an in-house pipeline. Despite a prior hypothesis that our patient had a mitochondrial disorder, we identified a causal variant in KCTD7 (p.Leu108Met) which provided a diagnosis of progressive myoclonic epilepsy type 3. This enabled us to reflect on our prior hypothesis and infer the cause of his Fanconi syndrome to be secondary to valproate therapy.

PS11.091
Congenital diaphragmatic hernia: a rare association with Nager syndrome due to SF3B4 mutation?

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Nager syndrome is a rare congenital malformation syndrome resulting from hypoplasia of the first and second branchial arches. It is a form of acrofacial dysostosis characterized by craniofacial and limb anomalies. Mutations in the SF3B4 gene have been confirmed as the cause of this condition in most affected individuals.
We report the case of a female neonate with an antenatal diagnosis of diaphragmatic hernia, severe micrognathia and polyhydramnios. At birth additional features of cleft palate and ventricular septal defect were noted. The severe micrognathia was associated with temporomandibular joint ankylosis requiring a tracheostomy. Other salient craniofacial features included low set ears with bilaterally small external auditory meatus and down slanting palpebral fissures. No significant upper limb abnormalities were detected.
A previously unreported heterozygous frameshift mutation (c.1175dupC) in exon 6 of the SF3B4 gene was identified by exome sequencing, confirming a diagnosis of Nager syndrome.
Diaphragmatic hernia has been described in only three previous cases of Nager syndrome, two of which had a confirmed molecular diagnosis. Therefore, this case adds evidence to suggest that diaphragmatic hernia should be considered to be a rare feature of the condition. In addition, our case is unusual in exhibiting no significant upper limb anomalies. To date the majority of cases of Nager syndrome have been reported with thumb hypoplasia/

aplasia. Therefore, this case highlights a milder upper limb phenotype. This case contributes to the understanding of the fetal and neonatal phenotype of Nager syndrome.

PM11.092

Clinical, immunological and genetic characteristics in a family with Nijmegen breakage syndrome

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Nijmegen breakage syndrome (NBS) is an autosomal recessive disease caused by mutations in the NBS 1 gene, located on chromosome 8q21. The gene product, nibrin is involved in DNA double strand break repair. The vast majority of NBS patients are of Slavic origin and share the founder NBS1 mutation (657D5). Essential features found in NBS were microcephaly, typical face, immunodeficiency, chromosomal instability, and a highly increased risk for malignancies.

We report three siblings with NBS, born to nonconsanguineous parents. The patients presented with obvious microcephaly, bird-like face, café au lait spots and normal intelligence. Cytogenetic analysis of children and healthy parents showed increased spontaneous chromosome breakage. The majority of the breaks involved four fragile sites on chromosomes 7 and 14 - 7p15, 7q36, 14q12, and 14q32. Immunological studies demonstrated a marked decrease in both B and T cell number and function. Mutation screening of the NBS1 gene revealed that children were homozygous for the 657del5 allele in exon 6, their parents are heterozygous for the same mutation.

First child died at 8 years of age from pneumonia. Second child had no history of recurrent infections. Third child had recurrent gastrointestinal and pulmonary infections since birth.

In conclusion patients with the same NBS1 genotype may show a variety of phenotypes. Other gene/epigenetic factors seem to play a role in phenotype presentation.

PS11.093

p.Arg1809Cys substitution in neurofibromin is associated with a distinctive NF1 phenotype without neurofibromas

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Analysis of 786 NF1 mutation-positive subjects with clinical diagnosis of neurofibromatosis type 1 (NF1) allowed to identify the heterozygous c.5425C4T missense variant (p.Arg1809Cys) in six (0.7%) unrelated probands (three familial and three sporadic cases), all exhibiting a mild form of disease. Detailed clinical characterization of these subjects and other eight affected relatives showed that all individuals had multiple cafè-au-lait spots, frequently associated with skinfold freckling, but absence of discrete cutaneous or plexiform neurofibromas, Lisch nodules, typical NF1 osseous lesions or symptomatic optic gliomas. Facial features in half of the individuals were suggestive of Noonan syndrome. Our finding and revision of the literature consistently indicate that the c.5425C4T change is associated with a distinctive, mild form of NF1, providing new data with direct impact on genetic counseling and patient management.

PM11.094

Clinical variability in a family with a novel SOS1 variant correlating with Noonan syndrome phenotype

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Noonan syndrome (NS) is a relatively common RASopathy caused by activating mutations in 14 genes associated with the Ras/MAPK signal transduction cascade. *SOS1* is one of the major NS-related genes, underlying the disease in about 10-13% of affected individuals.

We present a familial case of a NS patient, her affected brother and father with a novel heterozygous variant c.2013T>A (p.Ser671Arg) identified in exon 12 of *SOS1*, located in the RAS-exchange motif (REM) domain. The Mutation Taster prediction algorithm defined this variant as probably damaging, additionally, substitution segregated with the disease in the family.

The female patient was assessed by a clinical geneticist at the age of 5.5 years due to a 'peculiar' facial appearance in a child with a heart murmur. Her height and OFC were at the 25th centile and she presented with a distinctive phenotype suggesting NS: high and broad forehead, hypertelorism, sparse eyebrows, posteriorly rotated ears, curly scalp hair, broad chest, and hyperkeratotic palmar skin. No congenital cardiac anomalies were found on echocardiography. Additionally, electrocardiography did not show arrhythmia or hypertrophy, however heart enlargement was documented on a chest X-ray (index 0.55). Her affected father and brother exhibited anthropometric characteristics similar to the probant, however the boy was discordant for facial features and had straight hair.

Members of the reported family did not display clinical manifestations frequently observed in *SOS1* mutation-positive individuals (i.e. congenital heart defects, macrocephaly), but all three had mild cognitive deficits, which are a feature less commonly correlated with this gene.

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PS11.095

High-resolution genome wide SNP array analysis in patients with oculo-auriculo vertebral spectrum

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Oculo auriculo vertebral spectrum (OAVS) is a rare congenital disease characterized by aural, oral, mandibular and vertebral defects often associated with cardiac, pulmonary, renal, skeletal, and central nervous systems anomalies. The etiology of OAVS is still unknown. A number of chromosomal abnormalities containing genes potentially involved in the disease pathogenesis has been described. In the current study, we used a high-density Affymetrix SNP 6.0 array platform to evaluate the contribution to OAVS of rare submicroscopic chromosomal anomalies. Minimal diagnostic criteria for inclusion in the study were the presence of at least three of the following clinical characteristics: hemifacial microsomia, ear anomalies and preauricular pits or tags. A total of 60 subjects (34 females and 26 males) including 55 sporadic and 5 familial cases were studied. Thirty rare copy number variants (CNVs), ranging from 100 Kb to 1 Mb, were identified in 27/60 individuals. Of these, four had de novo origin in sporadic patients, whereas thirteen had been inherited from an unaffected parent. None of detected CNVs was listed in the database of genomic variants as well as in 80 ethnically matched Caucasian control subjects. Despite none of the identified CNV was recurrent, three independent chromosomal aberrations contained genes involved in the Pax-Six-Eya-Dach regulatory network. Current results suggest that individually rare CNVs are collectively significant contributors to the genetics of OAVS. Moreover, the CNVs detected in the present study suggest a number of OAVS candidate genes that warrant further investigation.

PM11.096

Novel syndromal obesity genes identified by custom targeted Next-Generation-Sequencing of 255 (candidate) genes.

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Syndromic obesity is defined by obesity in association with intellectual deficit and/or other congenital anomalies. An underlying genetic cause has not been identified in the majority of affected patients.
The recent developments of Next-Generation-Sequencing techniques provide a time- and cost efficient method to identify new obesity genes. We developed a custom NGS enrichment probe set, the 'Obesitome kit V1', aimed at enrichment and sequencing of 582 obesity related genes. Genes were selected on (presumed) obesity relevance, based on information from multiple publically available databases, pathway resources and scientific publications.
Based on the initial results of 55 morbid obese syndromal cases, we subsequently refined our enrichment to include the 255 most interesting obesity related genes ('Obesitome kit V2'). Of these 255 genes, 53 are routinely offered for NGS analysis in our DNA diagnostics section, the remaining genes can be used for extended research analysis.
Here we present clinical, molecular and functional follow-up study results of the first cohort (n=450) of (syndromal) morbid obesity patients. We identified known and novel pathogenic obesity gene mutations in approximately 15% of the investigated patients. These results will increase our understanding of the pathophysiological mechanisms of obesity and ultimately lead to personalized medicine of morbid obesity.

PS11.097
Identification of a novel truncating mutation in the distal part of KAT6B exon 18 in a girl with a typical Say-Barber-Biesecker-Young-Simpson phenotype

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Among a group of disorders collectively known as the blepharophimosis-mental retardation syndromes, the most distinctive phenotype is presented by patients with Say-Barber-Biesecker-Young-Simpson syndrome (SBBYSS).
SBBYSS is a rare multiple congenital anomaly syndrome characterized by a distinctive facial appearance (severe blepharophimosis, immobile mask-like face, bulbous nasal tip, small mouth with a thin upper lip), skeletal problems (joint laxity, long thumbs and great toes, dislocated or hypoplastic patellae), hypothyroidism, and global developmental delay. Recent studies demonstrate that *de novo KAT6B* mutations causing SBBYSS occur either throughout the gene, or cluster in a more distal (3') region of exon 18.
We present a 16-year-old girl with the typical, clinically recognizable pattern of SBBYSS features and a novel c.5819delA (p.Gln1940Argfs*11) molecular variant of *de novo* origin. The deletion is within the highly conserved C-terminal region of *KAT6B* and causes frameshift and occurrence of premature stop codon.
Our patient with moderate psychomotor delay, hypotonia, hypothyroidism, dislocated patellae and long thumbs also presents all of the facial features described so far in SBBYSS.
Our data contribute to the current knowledge on allelic heterogeneity observed at the *KAT6B* locus in classical SBBYSS and provide further evidence on the genotype–phenotype correlation in this rare disorder, expanding the locus-specific mutation database established so far. Dysmorphologists should keep this syndrome in mind during evaluation of patients with blepharophimosis and mild mental retardation, as the likelihood of identifying a *KAT6B* mutation in individuals with typical SBBYSS is high.
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PM11.098
An atypical presentation of OFD1 syndrome

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Introduction: Morning glory disc anomaly (MGDA) is a congenital abnormality of the optic disc reported as an isolated condition or associated with various anomalies including basal encephalocele and moyamoya disease. However the co-occurrence of these three entities is rare and the pathogenesis is still poorly understood. On the contrary Oral-Facial-Digital Syndrome type 1 (OFD1; OMIM 311200) is a well recognized X-linked-dominant developmental disorder belonging to ciliopathies. Among brain structural anomalies in OFD1, both sphenoidal encephalocele and moyamoya disease have never been reported.
Materials and Methods: The only child showed bilateral MGDA and coloboma, sphenoidal encephalocele, moyamoya disease and mild clinical features of OFD1.
Results: Sequence analysis of *OFD1* gene detected a *novel* hemizygous missense variant, c.1081T>C, resulting in p.Tyr361His mutation change.
Conclusions: Similarly to other ciliopathies, inactivation of *Ofd1* transcript is associated with defective Sonic hedgehog (Shh) pathway. *Ofd1* inactivation in zebrafish produces several malformations including retinal coloboma, whereas in mutant mouse it exerts a crucial role in developing of forebrain through the regulation of Shh signaling. On the other hand Shh is a critical pathway yielding a pleiotropic effects in many developmental processes such as in modulating the closure of the buccohypophyseal canal and the vessel formation. Here we report the first case of bilateral MGDA and coloboma, sphenoidal encephalocele and moyamoya disease due to a *novel* missense mutation in *OFD1* gene contributing to expand the phenotype of OFD1. Moreover we speculate on a possible pathogenic role of *OFD1* gene in patients with moyamoya disease, basal encephalocele and MGDA.

PS11.099
Haploinsufficiency of PCSK1 in a father and a son without obesity

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The PCSK1 gene was within the first genes implicated in the literature in severe early-onset obesity. Autosomal recessive congenital PCSK1 deficiency was shown to cause neonatal diarrhea and midchildhood severe obesity. Recently Philippe et al. (2014) also reported nonsense mutations in PCSK1 likely causing dominantly inherited human obesity.
We now report on a father and son with haploinsuffiency of PCSK1 without obesity. The 2 year-old boy was referred to our outpatient clinic because of psychomotor developmental delay. In the boy and his father we detected the microscopically balanced translocation : 46,XY, t(5;12)(q15;p13.1). By chromosomal microarray analyses (CMA) there was a deletion of about 157kb in the chromosomal band 5q15 encompassing the PCSK1 gene. The translocation was de novo in the father with behavioural problems in school and epileptic seizures in childhood until puberty. When we saw the boy at the age of 17 months his measurements were within the normal percentiles. The paternal grandmother reported the psychomotor development of the boy as comparable with the one his father had. The BMI of the father at the age of 40 was 25.2 kg/m^2.
The only gene deleted by CMA in this family was PCSK1. This gene is up to now not reported to be involved in developmental delay. The breakpoint on chromosome 12p13, however, may be responsible for the borderline developmental delay in this family: possibly involved gene: GRIN2B (12p13.1, mental retardation, autosomal dominant 6).
In our family there was a haploinsufficiency of PCSK1 and definitively no obesity supporting recessive inheritance of obesity in PCSK1 defects.

PM11.100
Penta X syndrome diagnosed in a newborn girl

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We present a detailed prenatal and postnatal clinical description and extended DNA analyses in a case of Penta X syndrome diagnosed in a newborn girl.
The pregnancy was uneventful until gestational age (GA) week 28, there were normal fetal ultrasonographic examination at GA 13+1 weeks, and no abnormalities diagnosed at the 20 weeks prenatal anomaly scan. At week 28 polyhydramnios was suspected leading to extra prenatal scans. Which revealed polyhydramnios, dilated intestines, suggesting possible intestinal atresia, IUGR with -23,1% to -30,7% weight deviation and short femora (-2,8 SD). Because of suspected brain sparing the birth was initiated at GA 38+3 weeks and the girl was born with a birth weight of 2,484 g.
She had hypertelorism, abnormal configuration of the ears, cleft palate (in the soft palate), bilateral single transverse palmar creases, bilateral clinodactyly, short lower limbs compared to the rest of the body, mild hypotonia and a shrill animal like cry. Echocardiography showed mild stenosis of the right pulmonary artery and mild coarctation of aorta, none of which were thought to have clinical significance.
Quantitative fluorescent polymerase chain reaction (QF-PCR) was performed on DNA extracted from a peripheral blood sample and showed a diallelic pentasomic pattern in 2 of 3 markers on the X-chromosome. The third marker was borderline within normal range. Chromosome analysis with Q-

banding was performed on cultured peripheral blood lymphocytes from the patient and revealed the karyotype 49,XXXXX in all examined metaphases. Results of the extensive molecular analyses including parental analyses and SNP-array will be presented.

PS11.101

Clinical and molecular characterisation of Pierre Robin Sequence with additional anomalies ("PRS-Plus")

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Pierre Robin Sequence (PRS) comprises micrognathia, cleft palate, glossoptosis and upper airway obstruction affecting 1 in 6000 neonates, often resulting in airway and feeding difficulties. Although syndromic forms of Pierre Robin Sequence are well known, the full phenotypic spectrum of non-syndromic PRS is less defined. We sought to provide an accurate phenotypic characterisation of a large cohort of 141 non-syndromic Pierre Robin Sequence patients managed at the Royal Children's Hospital in Melbourne from 1985 to 2012. We categorised patients into either "Isolated PRS" (n=83) or "PRS-Plus" (n=58) groups. Patients in the PRS-Plus group had additional anomalies beyond the craniofacial system, with the musculoskeletal and ocular systems most commonly involved. Choanal stenosis/atresia was the single most common co-existing craniofacial malformation. Compared to those with isolated PRS, PRS-Plus patients had worse outcomes at birth and during the neonatal period, with a higher proportion being born small-for-gestational-age, have failure to thrive and require surgical intervention for airway and feeding. A subset of patients with a family history of cleft and/or a musculoskeletal anomaly was selected for targeted DNA sequencing of the SOX9 conserved non-coding elements (CNEs) at chromosome 17q24. In one patient with PRS, talipes equinovarus and pectus excavatum, a single nucleotide substitution was identified in CNE1, at a consensus GATA1 transcription factor binding site; however the functional significance of this variant is currently being investigated.

PM11.102

Somatic mosaicism for the mutation in PIK3CA and significant activation of Akt/mTOR signaling in a girl with segmental overgrowth; Clinical report of PROS

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Here, we report the case of a girl who presented with hyperplasia of the lower extremities, macrodactyly, and multiple lipomatosis. We made a clinical diagnosis of PIK3CA-related overgrowth, which was subsequently confirmed by direct gene sequencing. The patient was born to healthy unrelated parents at 39 weeks of gestation. At birth, she presented with enlarged syndactyl toes, a broad foot, and a mass in her inguinal region. Her feet rapidly increased in size to almost twice the average size, and more so for her left foot. Adipose tissue was reduced in her upper extremities and torso. She underwent surgery several times to reduce adipose tissue in order to manage overgrowth. The patient's phenotype differed from that of Proteus syndrome, Klippel-Trénaunay-Weber syndrome, and CLOVES syndrome as follows. Her facial appearance was symmetric and nondysmorphic, overgrowth was limited to the lower extremities, her skin showed no nevi or hemangiomas, and no cerebriform skin was observed on hypertrophic regions (characteristic of Proteus syndrome). Histological analysis of the resected tissue revealed mature adipose tissue of normal morphology. We identified the patient as having the recently proposed clinical entity PROS (PIK3CA-related overgrowth spectrum). Using direct sequencing, a PIK3CA mutation (p.His1047Arg) was identified in DNA obtained from dissected hyperplasic adipose tissue; this was absent in DNA obtained from her blood lymphocytes. Significant activation of Akt/mTOR signaling was also observed in the patient's adipose tissue. This report contributes to the dysmorphological diagnosis of the phenotypic spectrum of mosaic PIK3CA mutations.

PS11.103

Testing strategies for PIK3CA-associated overgrowth disorders.

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Mutations in the PIK3CA gene have been found to cause several conditions related to overgrowth of tissues. These conditions include disorders of the brain such as Megalencephaly-Capillary malformation syndrome (MCAP) and hemimegalencephaly as well as segmental body overgrowth disorders like fibroadipose hyperplasia or Congenital, Lipomatous, Overgrowth, Vascular Malformations, Epidermal Nevi and Spinal/Skeletal Anomalies and/or Scoliosis syndrome (CLOVE). PIK3CA-associated disorders are caused by postzygotic activating mutations which are present in the mosaic form primarily in affected tissues of the body. The most frequent somatic mutations in PIK3CA identified in overgrowth syndromes occur in exons 9 and 20 (occasionally referred to as exons 10 and 21).
We routinely sequence exons 9 and 20 of PIK3CA using both Sanger and Next Generation Sequencing (NGS) methodologies. Here, we present our diagnostic strategy for a patient referred with clinical features suggestive of MCAP syndrome. Molecular analysis performed on DNA extracted from blood of the MCAP patient has produced results which required subsequent evaluation. Identification of a low-level somatic mutation is challenging and may require more than one tissue to be tested. Moreover lack of detection of a mutation in PIK3CA gene does not exclude the clinical diagnosis of PIK3CA-associated disorders due to limitations in detecting mosaicism from different tissue samples.
Results of further investigation of this case together with the potential role of NGS technologies in a diagnostic strategy for mosaic disorders will be presented.

PS11.105

Trisomy of 14q32 in a patient with Prader-Willi Syndrome-like phenotype

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Introduction: The main clinical features of Prader-Willi Syndrome (PWS) are hypotonia, hyperphagia, obsesity, hypogonadism, short stature, small hands and feet, mental disabilities, and behavioral problems. Signs and symptoms of PWS could also be found in patients who show other types of chromosomal abnormalities, among others Temple Syndrome (mat14UPD).
Materials and Methods: We report a case referred for genetic study with clinical suspicious of PWS due to the presence of hypotonia, hyperphagia, mood fluctuations, temper tantrums and developemental delay. 15q11 MS-MLPA test was normal so 14q32 MS-MLPA was carried out. Microsatellites of 14q32 region, CGH array and FISH study were analysed. Finally, deep genome sequencing of the 14q32 region was performed.
Results: The patient showed a gain of methylation and copy at MEG3-DMR. The microsatellites study revealed that the patient presented both paternal and one maternal chromosomes. FISH study of the patient pointed out that added copy could have been incorporated in tandem. CGH array and deep sequencing of the region revealed the exact boundaries of the insertion of the genomic extra material. Parents showed no (epi)genetics alterations of this region, so de novo origin was inferred.
Conclusions: We report a patient with an interstitial trisomy of 14q resulting from the insertion of part of 14q32 region of the paternal allele. It seems that the rearranged genomic segment was flanked by homologous low copy repeat (LCR) structures. These LCR could have acted as recombination substrates for non-allelic homologous recombination, leading to the gain of dosage of the region analyzed.
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PM11.106

Primary amenorrhea, visual impairment and intellectual disability in a girl with a complex rearrangement involving 5q33.3 and 9q21.2 microdeletions

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Case: We report a 21 year-old girl with primary amenorrhea, speech delay, mild intellectual disability, visual impairment, clinodactyly and low hairline. Total testosterone and 17 OH-progesteron levels were slightly increased. The other hormonal profile, brain MRI and pelvic USG were normal.
Methods and results: A cytogenetically balanced de novo complex chromosome rearrangement between chromosome 5 and 9 was detected by GTG and multicolor banding (MCB) techniques: 46,XX,der(5)(5pter->5q13.3::5q33-

>5q13.3::9q22.3->9q21.2::5q33->5qter),der(9)
(pter->q21.2::q22.3->qter) dn. Affymetrix 2.7K SNP array revealed 0.98 Mb and 0.94 Mb deletions on two of the breakpoint regions 5q33.3 and 9q21.2, respectively: arr 5q33.3(158,709,846-159,688,769)x1,9q21.2(79,573,426-80,513,997)x1. Completely deleted genes were IL12B, ADRA1B, TTC1, PWWP2A, FABP6, FOXB2, VPS13A, GNA14 and the disrupted genes were UBLCP1, CCNJL and GNAQ in these regions.
Conclusions: Somatic mosaic mutations of GNAQ cause Sturge-Weber Syndrome which may include intellectual disability. In DECIPHER, intellectual disability and speech delay have been reported in many patients with larger deletions on 9q21 overlapping with FOXB2, VPS13A, GNA14, GNAQ and in a patient with a deletion involving last exon of GNA14 and first two exons of GNAQ. Thus, disruption or haploinsufficiency of GNAQ and GNA14 may be related to intellectual disability. In addition, these changes may also be associated with primary amenorrhea and visual loss, because GNAQ and GNA14 play role in GnRH, estrogen, relaxin, androgen, serotonin and visual signaling pathways. However, it should be considered that the other breakpoints may also contribute to the phenotype.

PS11.107

Efficient clearance of progerin through autophagy induction and SRSF-1 downregulation under MG132 treatment in Hutchinson-Gilford progeria syndrome

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Hutchinson-Gilford progeria syndrome (HGPS; OMIM #176670) is an extremely rare premature and accelerated aging disease which affects 1 in 4-8 million children with symptoms resembling normal adult aging, including growth impairment, thin skin, loss of subcutaneous fat, alopecia, osteoporosis and cardiovascular disease leading to shortened lifespan and death at a mean age of 13.5 years. HGPS is caused by a de novo point mutation (c.1824 C>T, p.G608G) within exon 11 of the LMNA gene encoding A-type lamins. This mutation activates a cryptic donor splice site in exon 11 that leads to the deletion of 50 amino acids at its carboxy-terminal domain, resulting in a truncated and permanently farnesylated prelamin A called progerin. Accumulation of progerin is a hallmark of HGPS that affects the integrity of the nuclear scaffold, leading to nuclear blebbing and functional defects in cultured cells. We show that progerin is sequestered, together with other proteins (lamins B1/B2, emerin), into abnormally shaped Promyelocytic-Nuclear Bodies (PML-NB), identified as novel biomarkers in Progeria. MG132, inhibiting proteasome activity, induces progerin nucleocytoplasmic translocation after a transition through the nucleolus, and progerin degradation through macroautophagy. MG132 also strongly reduces progerin production through caspase-linked cleavage of SRSF-1 controlling prelamin A mRNA splicing. In vivo, through MG132 treatment, progerin expression decreases in skeletal muscle from LmnaG609G/G609G mice. Altogether, we demonstrate progerin clearance based on MG132 dual action and shed the light on a promising class of molecules towards an encouraging therapy for Progeria and related diseases.

PM11.108

Clinical manifestations of germline PTEN pathogenic mutations: A review of published cases with emphasis on phenotypic traits

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Background: Cowden syndrome is an autosomal dominantly inherited tumour predisposition syndrome with a prevalence around 1/200 000 in the European/USA population. It is associated with increased risk of developing malignant tumors including breast, thyroid and endometrial cancer. Germline mutations in PTEN gene are considered an underlying cause of the disease. However, 37% of mutation carriers do not fulfill clinical diagnostic criteria for any defined syndrome, and therefore a wider phenotype could be associated with pathologic mutations in PTEN.
Purpose: This study aimed to describe the comprehensive clinical phenotype associated with PTEN mutations, to examine potential genotype/phenotype correlations, and to evaluate the relevance of currently used clinical diagnostic criteria.
Method: A systematic review of published cases and observational articles was undertaken and clinical characteristics of described cases were compiled.
Results: 297 articles and abstracts describing 682 presumably unique individuals were identified in the study. Clinical traits included in established criteria for PTEN related syndromes occurred with high frequency. Patients with germline PTEN mutation displayed mixed polyposis as well as vascular malformations, and several novel traits were noted similar to observations seen in mouse models of PTEN deficiency. Germ cell/gonadal malignancies were significantly more prevalent among the study population compared to general population. Further, Cowden syndrome appeared to be diagnosed more frequently among females, and Bannayan-Riley-Ruvalcaba syndrome more frequently among males, potentially resulting from gender weighting in the diagnostic criteria.
Conclusion: The phenotype associated with PTEN mutations seem to be wider than believed, and further study is needed for understanding the prevalence of novel clinical characteristics among mutation carriers and their ramifications.

PS11.109

New mutations associated with Rasopathies identified by Sanger and next generation sequencing in a Central European population

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Introduction
Rasopathies represent a group of dysmorphic syndromes caused by mutations in RAS/MAPK signaling pathway, such as Noonan syndrome, Noonan syndrome with multiple lentigo, Costello, cardio-facio-cutaneous and neurofibromatosis-Noonan syndrome. These syndromes share clinical features including mainly short stature, specific cranio-facial features and heart defects. Therefor the diagnosis of Rasopathies is a difficult task.
Aim
Determination of genetic cause and description of genotype-phenotype correlation in patients with Rasopathy phenotype by Sanger and next generation sequencing.
Patients and methods
Mutation analysis of 14 genes associated with Rasopathies (PTPN11, SOS1, RAF1, BRAF, KRAS, HRAS, NRAS, MAP2K1, MAP2K2, SHOC2, CBL, NF1, RIT1 and SPRED1) was performed by Sanger and target sequencing using the HaloPlexTM Target Enrichment System on MiSeq sequencing platform in 48 patients from Central Europe - Slovakia (18), Slovenia (20), Hungary (3), Czech republic (2) and Austria (5). Patients were selected according to the scoring questionnaires oriented to the main clinical features of Rasopathies. Data were analyzed by ChromasPro and SureCall softwares.
Results
We identified mutations in 60% of examined patients (29/48) including 3 new variants (c.87+2T>C in SOS1, c.1175G>A in BRAF, c.490C>A in MAP2K1 genes) with potentially pathogenic effect on patient´s phenotype.
Conclusion
We contributed to enlargement of the mutation spectrum occuring in patients with Rasopathies and described the possible mutation effect on the phenotype.
This work was supported by Ministry of Health of the Slovak Republic under the project registration number
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PM11.110

Molecular findings in Spanish patient with RASopathies. Extending the analysis with prioritization methods

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Introduction: RASopathies are caused by germline mutations in genes encoding protein components of the Ras-MAPK pathway.
Material and methods: First, 12 genes (*PTPN11, SOS1, RAF1, KRAS, SHOC2, NRAS, BRAF, MAP2K1, MAP2K2, HRAS, LRP1, CBL*) associated with the Ras-MAPK pathway were studied by next-generation sequencing (NGS) in 14 Spanish patients with clinical suspicion of RASopathies. A segregation test

was performanced in the patients' parents when they were available. Additional candidate genes were selected with an R custom script using PSIQUIQ library (Shannon P, 2015).

Results: In 3 patients, clinical diagnosis was molecularly confirmed in the first screening. Two cases are waiting for segregation study.

Using the script, more than 1000 candidate genes were obtained, based on several molecular interaction databases, using as query the previous 12 genes. Twenty-three genes with more than 5 interactions with query genes and/or more than 25 interaction methods reported, and availability in NGS capture kit used, were selected for the second screening. This additional screening revealed some variants of uncertain clinical significance (VUCS) in 8 patients.

Table 1. Heterozygous changes identified in patients samples.

Patient	Phenotype	1st screening*	Mutation origin	Clinical significance	2nd screening*
1	NS	*RAF1*: c.785A>G	No data	VUCS. Novel	*JAK3*: c.2164G>A *ERBB2*: c.1157C>A
2	NS	*PTPN11*: c.236A>G	No data	Pathogenic	*PIK3CG*: c.1076G>A *PIK3CG*: c.2496T>G
3	NS	*SHOC2*: c.74A>G	No data	VUCS. Novel	Negative
4	CFC	*MAP2K2*: c.181A>G	de *novo*	Pathogenic	Negative
5	NS	*SOS1*: c.806T>G	Affected mother	Pathogenic	*IL2RA*: c.76G>C (Homozygous)
6	NS	*SOS1*: c.1964C>T	Unaffected father	Benign	Negative
7	NS	*SOS1*: c.1964C>T	No data	Benign	*CRKL*: c.634A>C
8	NS	Negative	-	-	*LRP1*: c.7636G>A
9	NS	Negative	-	-	Negative
10	NS	Negative	-	-	Negative
11	NS	Negative	-	-	*CD2AP*: c.1632+3G>A
12	NS	Negative	-	-	*CD2AP*: c.1632+8G>T *ERBB2*: c.1157C>A *PIK3CB*: c.2150A>G
13	NS	Negative	-	-	Negative
14	NS	Negative	-	-	*JAK3*: c.452C>G

NS- Noonan syndrome.
CFC- Cardiofaciocutaneous syndrome.
* Transcripts according to HGMD.

Conclusion: Multi-gene analysis by NGS has improved the molecular diagnosis of RASopathies, but many cases still remain undiagnosed. Bioinformatics tools can propose new candidate genes related to these disorders as shown, but further studies are needed to confirm their causal effect.

PS11.111

Ring chromosome 22 in patients with multiple congenital anomalies

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Introduction: Ring 22 chromosome is a rare chromosomal aberration, with only ~100 cases reported and no consistent clinical picture. We present 3 cases with variable clinical phenotypes: RPM, microcephaly, hypotonia, ataxia.

Materials and Methods: Chromosomal analysis was done from cultured cells of PHA-stimulated lymphocytes from blood and cultured skin fibroblasts using GTG banding. Specific characterization of marker chromosome was done using multicolor fluorescent in situ hybridization (M-FISH).

Results: Two patients had aberrant karyotypes: 46,XX,r(22)(p12q13) and 46,XY,r(22)(p12q13), respectively. Third patient had aberrant mosaic karyotype: mos 46,XY,r(22)(p12q13)[44]/46,XY,dic r(22)(p12q13[6]). Cytogenetic analysis from this patient's skin fibroblasts showed presence of only one clone: 46,XY,r(22)(p12q13)[52]. Clinical features in patients with ring22 can be associated with 22q13.3 syndrome (Phelan McDermid syndrome) since the formation of ring22 is followed by terminal deletion of p and q arms. The last described patient had cells with dic r(22) causing presence of three copies of 22p12-22q13.2 region in minority (6/50) of peripheral blood lymphocytes. This aberration still might account for the CES (cat eye syndrome) features since it may have been present at a much higher frequency early in development and then subsequently lost.

Conclusion: Our report emphasizes the importance of cytogenetic and molecular-cytogenetic approach in order to better understand the spectrum of abnormalities in each case, and assess genotype-phenotype correlation.

PM11.112

The significance of RIT1 mutations in the etiology of Noonan syndrome: phenotype-genotype correlations in Polish patients

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Noonan syndrome (NS), a RASopathy which exhibits genetic and phenotypic heterogeneity, has been associated with germline mutations in 14 genes related to the RAS/MAPK signaling pathway, including recently discovered *RIT1* gene encoding a small GTPase from the RAS superfamily.

We present 6 new unrelated NS patients of Polish origin with 5 different pathogenic substitutions in conserved domains of RIT1: p.Ser35Thr, p.Ala57Gly, p.Ala77Ser, p.Phe82Leu, p.Gly95Ala. *De novo* occurrence has been confirmed in 4 cases, whereas p.Ala77Ser was identified in the patient's father, who also presented with NS phenotype. In our molecularly defined group of NS patients *RIT1* mutations constitute ~8% (6/76).

The clinical findings of our study largely agree with the literature, as the patients exhibited typical NS features, however, without intellectual disability, additionally, only 2/7 had short stature. It is worth noting that 4/6 patients suffered from postnatal lymphedema and other lymphatic disorders. Cardiac anomalies occurred in 4/7 individuals. The patient with p.Phe82Leu mutation died early due to the severe hypertrophic cardiomyopathy. In the affected father with p.Ala77Ser substitution teratoma maturum testis has been identified.

Our results, together with the recently published data concerning 4 other Polish individuals, introduce the largest group of NS patients of Caucasian origin with *RIT1* mutations. They contribute to the further delineation of molecular and clinical profile of *RIT1*-related NS and underline that *RIT1*, as the third most frequently affected gene in NS, should certainly be included into the diagnostic panels screening for RASopathy disorders.

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PM11.114

Expanding EP300 mutational spectrum of Rubinstein-Taybi syndrome and literature overview

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Rubinstein-Taybi syndrome (RSTS, #180849, #613684) is a rare congenital neurodevelopmental disorder characterized by postnatal growth deficiency, typical skeletal abnormalities, dysmorphic features and intellectual disability. Mutations in two genes, CREBBP (16p13.3) and EP300 (22q13.2), encoding two homologous transcriptional co-activators, have been identified in ~55% and ~8% of affected individuals, respectively.

To date, only 14 EP300-mutated RSTS patients have been described, six in our laboratory, and 12 additional mutations are reported in the LOVD database, summing up to 26 different alterations.

EP300 analysis of CREBBP-negative RSTS patients led to the identification of five novel further inactivating de novo mutations: an exons 17-19 deletion and two stop mutations in exons 23 and 28 respectively (c.3829A>T/p.K1276* and c.4585C>T/p.R1528*), a duplication in exon 29 (c.4640dupA/p.N1547Kfs*3) and a IVS9 splicing mutation (c.1878-12A>G).

RNA analysis on patients carrying the c.4585C>T/p.R1528* and c.4640dupA/p.N1547Kfs*3 mutations, which affect the KAT domain, revealed the presence of both the WT and the aberrant transcript.

All EP300-mutated patients show a convincing, although mild, RSTS phenotype including typical broad thumb and big toes, craniofacial dysmorphisms, prenatal and postnatal growth delay with a moderate to mild intellectual disability. Organ malformations were rare and, when present, always isolated.

This study, expanding the RSTS EP300 mutational spectrum to 31 alterations and providing a clinical description of five additional patients, will enhance RSTS-EP300 mutated patients hallmarks identification and improve the clinical practice allowing a better genotype-phenotype correlation doing.

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PS11.115

DOCK4 mutated in Seckel syndrome

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Seckel syndrome is a genetic heterogenic disorder caused by mutations in several genes involved in the regulation of the cell cycle and DNA repair. Mutations in each gene are extremely rare and only account for the diagnosis in single or a small number of families.
Two sisters from a Turkish consanguineous family presented with primary microcephaly (-8 SD), short stature of prenatal onset (-5 SD), behavioral issues, and intellectual disability.
Their facial features were suggestive of Seckel syndrome. Homozygosity mapping (using Affymetrix 6.0 SNP arrays) identified 5 regions; 1p34.2, 2q22.2-2q22.3, 3p24.1-p24.2, 7q31.1-q32.3 and 18q21.33q22.3. No known Seckel genes are located within these regions. With whole-genome sequencing (Complete Genomics) the homozygous *DOCK4* c.3470G>A variant was identified, leading to a putatively deleterious amino acid substitution (p.R1157H).
The variant was absent from dbSNP and 414 Mediterranean control chromosomes. *DOCK4, dedicator of cytokinesis 4,* encodes a Rac1 guanine exchange factor (GEF), which is involved in the PDGF-dependent cell migration through modeling of the actin cytoskeleton. DOCK4 is highly expressed in brain and during embryonic development and it has been proposed to play in role in neurite differentiation and extension. We stimulated fibroblasts from one of the affected individuals with PDGF-BB, and stained the fixated cells with the F- actin marker rhodamine phallodoidin. This showed a poorly organized actin network in patient fibroblasts compared to control cells. A PDGF-responsive migration assay on patient cells is pending. Confirmation of our data in additional families will encourage further studies on the role of this gene in human growth and brain development.

PM11.116

‚Serpentine-like syndrome' - a very rare multiple malformation syndrome characterised by brachioesophagus and vertebral anomalies

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"Serpentine-like syndrome" is a severe and rare multiple malformation syndrome characterised by brachioesophagus with secondary intrathoracic stomac associated with cervical (sometimes more extensive) congenital vertebral anomalies (failure of fusion, segmentation and formation, or combinations of these). Other associated anomalies have been described such as malposition and herniation of other abdominal organs.
We report the case of a baby girl born prematurely (29wog) due to polyhydramnios. She presented IUGR, craniofacial dysmorphism, median cleft lip and short neck. She had a midline diaphragmatic hernia, a very short oesophagus. The stomach, spleen and part of the pancreas were located in the thorax. She was also diagnosed with a large rachischisis from cervical to thoracic spine, with major defect of the anterior arches and a cyst of the spinal cord. The lobulation of the lungs was abnormal. Both the echocardiogram and the transfontanellar ultrasound were normal. Most of these malformations have been observed prenatally. SNP array was normal. She died at age 12 days. No relevant family history was registered.
To our knowledge, our patient represents the 7th report of a patient with "Serpentine-like syndrome". Brachioesophagus and congenital vertebral anomalies (in particular rachischisis) are the cardinal features of this multiple congenital malformations syndrome. All cases have been sporadic so far and the cause was not found in any of them. It is likely that this condition originates during early embryonic development. We believe that the specificity of the presentation as well as the similarities between cases suggests a common, yet to identify, genetic cause, probably involving developmental „toolkit" genes or related pathways.

PS11.117

Novel myopathy in a newborn with severe thoracic dysplasia caused by mutations in the SBDS-gene. Further delineation of the phenotypic spectrum of Shwachman-Diamond syndrome

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Shwachman-Diamond-Bodian syndrome (SDS) is a pleiotropic disease, in which the main features are bone marrow dysfunction and pancreatic insufficiency. Skeletal changes can occur in a variable degree and in rare cases as severe congenital thoracic dystrophy. We report on a new-born baby boy with asphyxia, narrow thorax and severe hypotonia initially suggesting a neuromuscular disease. The muscle biopsy showed myopathic changes with prominent variability in muscle fibre size and abnormal expression of developmental isoforms of myosin. The myofibrils showed focal loss and disorganization of myofilaments and thickening of the Z-discs including some abortive nemaline rods. The boy became permanently dependent on assisted ventilation. Pancreatic insufficiency has subsequently been evidenced explaining the malabsorption and failure to thrive. Except transitory thrombocytopenia and leukopenia, no other major hematologic abnormalities were noted. He had bilateral nephrocalcinosis with preserved renal function. Transitory liver dysfunction with increased transaminases and parenchymal changes on ultrasound were registered. The clinical diagnosis was molecularly confirmed by detection of compound heterozygous mutations in the SBDS gene using whole-exome sequencing: a recurrent intronic mutation causing aberrant splicing (c.258+2T>C) and a novel missense variant in a highly conserved codon (c.41A>G, p.Asn14Ser) considered to be damaging for the protein structure by in silico prediction programs. The parental carrier status has been confirmed. This case illustrates the challenges in differential diagnosis of pronounced neonatal hypotonia with asphyxia and highlights the muscular involvement in SDS. To our knowledge, this is the first report of evidenced myopathy in a patient with clinically and molecularly confirmed SDS.

PM11.118

Molecular analysis of a novel intragenic deletion in GPC3 in three cousins with Simpson-Golabi-Behmel syndrome

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Simpson-Golabi-Behmel syndrome (SGBS) is an X-linked recessive overgrowth syndrome, characterized by pre/postnatal overgrowth, distinctive craniofacial features, a broad spectrum of congenital malformations, intellectual disability (ID) of variable degree and an increased risk for embryonal tumors. SGBS is caused by deletions, duplications and point mutations in *GPC3*, encoding a membrane associated cell surface heparan sulfate proteoglycan named glypican 3. GPC3 plays essential roles in regulation of cell growth signaling and cell division. Several large intragenic deletions of *GPC3* detected by array-CGH and PCR analysis of genomic DNA but so far not on mRNA level have been reported. Here we report on a family with three affected cousins who display typical clinical features of SGBS, such as overgrowth, accessory nipples, hypertelorism and ID. By initial microarray-CGH we identified a deletion of approximately 30-50 kb that includes at least one exon of *GPC3*. By subsequent Sanger sequencing of genomic DNA we could map the chromosomal breaking points to define a deletion of 43,617 bp including exons 6 and 7 of the *GPC3* gene. RT-PCR analysis on RNA derived from whole blood could further confirm the deletion of both exons on transcript level. This loss of two exons results in a frameshift and a premature stop.
Based on our results we have established a breakpoint spanning PCR that could also identify the mutation in the mothers and grandmother of the patients. Thus we provide a molecular test that allows accurate genetic counselling and prenatal diagnosis for this family.

PS11.119

An unexpected diagnosis of Troyer syndrome in two brothers with a Silver-Russell syndrome-like phenotype

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Our laboratory received independent requests for UPD7 testing in two boys with clinical suspicion of Silver-Russell syndrome (SRS), a normal ICR1 methylation pattern of chromosome 11p15, normal karyotype; UPD7 was not

confirmed. The patients turned out to be brothers, sons of first-degree cousins from Morocco with irrelevant family history.
The sibs, 3 and 5 years-old, presented pre- and postnatal growth retardation, developmental delay with predominant speech impairment, hypotonia and joint hyperlaxity, happy demeanor but no clear walking difficulties. Physical evaluation showed relative macrocrania, prominent maxilla, pectus excavatum, flat feet.
Considering parental consanguinity and a difficult differential diagnosis, whole exome sequencing was performed, which led to the identification of a novel homozygous variant c.892dupA in the SPG20 gene in both affected sibs. This variant causes a premature stop codon in spartin, the protein encoded by SPG20. Parents are heterozygous carriers of this variant, that is absent from large control databases (ESP, ExAc).
Mutations in SPG20 have been reported in Troyer syndrome, an autosomal recessive disorder, mainly characterized by progressive spastic paraparesis, pseudobulbar palsy, distal amyotrophy, developmental delay, and short stature, with onset in early childhood.
A neurologic examination, performed after this finding, showed lower limb muscular hypotrophy with mild distal hypertonus and intra-rotated feet when running in both sibs.
The mutation therefore seems causative of the patients' phenotype.
Even if it is a rare condition, Troyer syndrome should be considered in patients with syndromic short stature (SRS-like) and developmental delay, for prognostic and counseling issues.

PM11.120

Potocki-Lupski syndrome due to a small supernumerary marker chromosome derived from chromosome 17

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Potocki-Lupski syndrome (PLS) is usually caused by the chromosome 17 duplications at 17p11.2. However, there are rare cases of this syndrome originating from chromosome rearrangements producing partial trisomy 17p encompassing the critical region. Here, we present a 4-year-old girl with a developmental delay, language impairment, symptomatic epilepsy, behavioral problems, congenital corneal opacity of the right eye, and dysmorphic features mimic PLS facial appearance. Cytogenetic analysis has revealed a supernumerary marker chromosome. Molecular cytogenetic analysis by SNP array molecular karyotyping and FISH has indicated this marker chromosome to be derived from chromosome 17. This small supernumerary marker chromosome (sSMC) was detected in about 60% of cells. SNP array and FISH showed approximately the same percentages of abnormal cells. Molecular karyotyping has shown that sSMC was composed of a genomic region corresponding to 17p11.2q11.1 (8.5 Mb) and affected 164 genes. sSMC was larger than commonly reported duplications (3.7 Mb). In the available literature, there have been only 3 reports describing supernumerary "marker" chromosomes 17 in PLS. Interestingly, the present case exhibited a derivative chromosome affecting larger genomic region as to those previously reported. The latter seems to be key explanation of clinical manifestations additional to PLS phenotype. Finally, this case exemplifies that sSMCs addressed through SNP array and FISH represent an attractive perspective for genotype-phenotype correlations. Supported by a grant from the Russian Science Foundation (#14-15-00411).

PS11.121

Supernumerary ring chromosome 7 in mosaic. Molecular cytogenetic analysis and clinical findings.

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Supernumerary ring chromosomes cause genomic imbalance and can be associated to an abnormal phenotype depending on the gene content. It is important to identify and characterize the extra chromosome composition in order to perform phenotype-genotype correlations. Only thirteen patients with a supernumerary pericentromeric ring (7) chromosome have been reported to date. Among them, language delay and poor expressive speech performance are frequently reported. We report a patient with a de novo supernumerary r(7) chromosome present in 56% of lymphocytes, found by conventional cytogenetic analysis in a 19 years-old girl referred for low IQ, disturbed behaviour and a peculiar facial phenotype. Subsequent array-CGH analysis detected a 25 MB duplication encompassing the pericentromeric region 7p11.2-7q21.11. Based on the array-CGH results, the gene content of the r(7) chromosome was determined. The 7q11.23 region included in the ring chromosome has been associated to the Williams-Beuren syndrome when deleted (OMIM #194050) and to the duplication 7q11.23 syndrome (OMIM #609757). This duplication causes a recognizable phenotype characterized by language delay and mild craniofacial anomalies, showing variable expressivity. In some cases, the genetic imbalance is inherited from one of the normal parents. Clinical assessment of our patient showed some minor facial dysmorphisms, such as a narrow flat front, almond-shaped palpebral fissures, small mouth, thin lips and pointed chin, besides a central obesity. Psychological tests disclosed an IQ in the low limit of normality, showing variable performances in different areas. Aggressive behaviour and low social skills were also noted. Phenotype-genotype associations are discussed.

PM11.122

Genetic Susceptibility of Thalidomide Teratogenesis: study of variants in developmental genes in humans

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Introduction: Thalidomide remains infamous for being a teratogen, causing severe damage to embryos exposed during early pregnancy. Remarkably Thalidomide is currently used to successfully treat conditions like Leprosy complications and Multiple Myeloma worldwide. Yet, surprisingly, how thalidomide caused teratogenesis is still not fully resolved. Many theories have been put forward, some of the widely accepted ones include induction of oxidative stress, angiogenesis inhibition and disruption of Cereblon E3 ubiquitin ligase complex which may alter molecular pathways involving developmental genes resulting in thalidomide embryopathy (TE). **Objective:** To identify if genetic variants of susceptibility to TE in developmental genes can be detected in subjects with TE. **Methods:** We compared the allelic and genotypic frequencies of single nucleotide polymorphisms (SNPs) in several important signalling molecules that have multiple roles in embryogenesis, specifically, *FGF8, FGF10, BMP4, SHH* and *TP53* genes in 28 TE subjects to 68 non-TE subjects from the Brazilian population without congenital malformations. All DNA samples were genotyped by Real-Time PCR and all the necessary ethical consents were obtained. **Results:** For the genes looked at in this study we did not identify differences in genetic frequencies between the TE and control group. **Conclusions:** We did not, at this time, identify genetic susceptibility to TE for the evaluated variants. However, we have only used a small sample size and a limited number of gene targets, to validate this approach. We are looking at other potential targets using the same methodology to help in understanding of molecular mechanism of teratogenesis. **Funding:** CNPq, CAPES and FIPE-HCPA.

PS11.123

Macrothrombocytopenia and developmental delay with a de novo CDC42 mutation: Yet another locus for thrombocytopenia and developmental delay

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The combinatory phenotype of thrombocytopenia and developmental delay has been described for two genetic conditions: a chromosome 11q deletion that is referred to as Jacobsen syndrome, and a 21q22 microdeletion syndrome. Herein, we report a young girl who presented with persistent macrothrombocytopenia and a developmental delay. A physical examination showed distinctive facial features with microcephaly. She communicated with simple words. She was able to identify colors and to name objects. She had an intention tremor and fell while attempting a tandem gait. Whole exome sequencing identified a de novo mutation in CDC42 (NM_001039802), i.e., c.191A>G, p.Tyr64Cys as the sole candidate in an autosomal dominant de novo mutation model or an autosomal recessive model. The mutation was confirmed using Sanger sequencing. CDC42 is a critical regulator of the cytoskeleton. While the observation may be coincidental, the strong degree of similarity between the phenotype of the proposita and that of mice lacking Cdc42 suggests a probable causal relationship. The conditional knock-out of Cdc42 in mice results in central nervous system defects and macrothrombo-

cytopenia. This pattern of hematologic indices was exactly the same as that which was observed in the proposita. We suggest that this CDC42 mutation may represent yet another mechanism leading to the combinatory phenotype of persistent macrothrombocytopenia and developmental delay.

PM11.124

TMEM-107 is anchored to ring-like subdomains of the transition zone (TZ) membrane and organizes the TZ recruitment of ciliopathy transmembrane proteins

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The ciliary transition zone (TZ) is thought to control ciliary formation, composition and signaling by facilitating a protein diffusion barrier at the ciliary base, and TZ defects are associated with ciliopathies such as Meckel-Gruber syndrome (MKS), Nephronophthisis (NPHP) and Joubert syndrome (JBTS). Here, using a co-expression/evolution profiling approach to uncover candidate TZ genes, TMEM107 was identified as a new TZ protein mutated in patients with oral-facial-digital syndrome type VI (OFD VI) and JBTS. Mechanistic studies in C. elegans roundworms reveal TMEM-107 functions redundantly with NPHP-4 to regulate cilium integrity, TZ docking and the assembly of TZ membrane-microtubule Y-link connectors. Consistent with evolutionary and mammalian biochemical analyses, TMEM-107 occupies an intermediate layer of the nematode TZ-localised MKS functional module and organises the TZ recruitment of a distinct transmembrane submodule including TMEM-17, TMEM-231 and JBTS-14/TMEM237. Furthermore, transmembrane MKS module proteins including TMEM-107 are immobile within the TZ membrane and display ring-like super resolution localisation patterns reminiscent of the ciliary necklace. Thus, TMEM107 is a ciliopathy constituent of an anchored ring-like domain of the TZ membrane and organises a discrete transmembrane component of the C. elegans MKS module.

PS11.125

Trisomy of the short arm of chromosome 10: description of three new cases

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Trisomy 10p is a rare, complex syndrome of mental retardation and multiple congenital malformations caused by the partial or total duplication of the short arm of chromosome 10. This abnormality may be a de novo occurrence or may be inherited from a carrier parent. Most cases appear because of an unbalanced segregation of famililal chromosomal translocations that result in trisomy 10p associated with other additional segmental imbalaces.
We describe three cases of non-mosaic trisomy 10p resulting from familial translocations: two sisters with a complex cytogenetic anomaly consisting in 10p trisomy and a rare subtelomeric 2p25.1 deletion and one other unrelated girl with 10p trisomy, without any apparent additional genomic imbalance (46,XX,der(2)t(2;10)(p25.1;p11.1)mat and, respectively, 46,XX,der(14)t(10;14)(p11.1;p11.1)mat). The inheritance and identity of the translocations were ascertained by extensive familial cytogenetic and FISH studies.
The few tens of patients with trisomy of the short arm of chromosome 10 reported to date have shown variable clinical manifestations. The clinical presentation for the girl with der(14) (10 days old) is very similar to that found for pure trisomy 10p reported in the literature, with facial dysmorphism, congenital abnormalities, hypotonia and severe developmental delay.
For the two sisters with der(2) (ages 1y 4mo and 8y 1mo), the phenotypic features slightly overlap those previously described for trisomy 10p, with additional clinical characteristics, probably due to the accompanying monosomy.
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PM11.126

Management of Children with Trisomy 13: Clinical Details of 22 Patients Receiving Intensive Treatment.

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Trisomy 13 (T13) is characterized by multiple congenital anomalies, severe developmental delay, and a short life span with the 1-year survival rate as 5-10 % and the median survival time as 7 days through population-based studies. Management of T13 is controversial, supposedly due to the lack of precise clinical information of this syndrome especially on efficacy of treatment. To delineate the natural history of T13 managed under intensive treatment, we reviewed detailed clinical data of 22 patients with full T13 admitted to Nagano Children's Hospital from 1994 to 2014.
Major clinical findings included congenital heart defects (91%), cleft lip or cleft palate (68%), polydactyly (59%), and cryptorchidism (50% of male). 19 patients received resuscitation by intubation and required Mechanical ventilation. Of these, six were extubated and 11 needed tracheostomy. A total of 29 surgical interventions except for cardiac surgery were performed on 15 patients. Enteral feeding was accomplished in 19, six of whom were fed orally. Eight patients could be discharged home. The survival rate at age 1 week, 1 month, and 1 year was 100%, 95%, and 41%, respectively. Median survival time was 271 days (range, 22–3987). Common final modes of death were congenital heart defects and heart failure (50 %). These data were similar to those obtained in patients with trisomy 18 in our hospital [Kosho et al., 2006].This study has demonstrated improved prognosis through intensive treatment, which would be helpful for clinicians to offer the best information on treatment options to families of patients with T13.

PS11.127

Discordant prenatal and postnatal cytogenetic findings in a case of de novo complete trisomy 5p

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Isolated trisomy of the complete short arm of chromosome 5 is a very rare constitutional chromosomal finding. We report a case of a meanwhile 6 month old dysmorphic girl with a prenatally not detected *de novo* trisomy of the complete short arm of chromosome 5.
Prenatal ultrasonography at 12th week of gestation revealed a dorsonuchal edema of 7.00 mm. Subsequently, chorionic villus sampling (CVS) was performed for conventional chromosomal analysis. After direct preparation the CV trophoblast cells showed a numerically normal female karyotype (46,XX), whereas after long-term culture all analysed CV mesenchymal core cells showed a numerically abnormal female karyotype with tetraploidy (92,XXXX).
Fetal ultrasonography at 17th week of gestation revealed persistent nuchal edema, bilateral clubfeet and a generally petite developed fetus. Parents rejected a recommended amniocentesis. Molecular analysis for Noonan-Syndrome and high-resolution array-CGH performed with DNA of the cultured tetraploide cells showed negative results.
Five weeks after birth the child was presented to a geneticist because of several dysmorphic features and a severe failure to thrive. Chromosomal analysis of peripheral blood showed an aberrant female karyotype with an additional marker chromosome segregating in all cells. The marker could be identified as an isolated complete short arm of chromosome 5 confirmed by molecular-cytogenetic analysis: 47,XX,+del(5)(q11.1).ish del(5)(wcp5+). The report will discuss the discordance of the results, describe the established dysmorphism and emphasize the importance for additional cytogenetic testing of a second fetal tissue, if abnormal prenatal ultrasound findings persist and cytogenetic analysis of one fetal tissue show negative or unclear results.

PM11.128

Molecular diagnosis of Czech tuberous sclerosis complex patients in the period 2009-2013

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AD inherited tuberose sclerosis complex, caused by TSC1 or TSC2 gene mutation, is characterised by hamartomatous growths in many organs (brain, heart, kidney etc.). Molecular diagnostics for Czech TSC patients is performed at Institute of Medical Genetics, University Hospital Olomouc since 1995. Previously used methods for unknown mutations detection (DGGE

and SSCP) have been replaced from 2009 by MLPA and Sanger sequencing of all TSC coding sequence, with the aim to increase the efficiency of causal mutations detection.
In the period from 2009 to 2014 the following file of patients has been investigated by our laboratory for the TSC genes analysis: 124 new cases of TSC (7 prenatal samples) and 37 patients indicated before the year 2009. The file also included family members (127 postnatal examinations and 14 prenatal samples) and confirmatory samples (45 examinations).
For the period a complete analysis of TSC genes was carried out in 98 index cases. 87 causal mutations were uncovered, 29 of them not previously published in TSC1/TSC2-LOVD database. 64 cases were related to the TSC2 gene mutation, 23 patients have TSC1 gene mutation, for 5 persons (3 prenatal samples from fetuses with rhabdomyoma) no mutation was found. In 6 individuals sequential change of unknown significance was found, to clarify its possible causality it is necessary to investigate the parents' DNA, or other members of the family.
Since 2014, we are implementing massive parallel sequencing (MPS, MiSeq) of both TSC genes in order to speed up the passage of the samples through the laboratory. Preliminary MPS results will be discussed.

PS11.129

A paternal isodisomy of chromosome 12 causes two different disorders in a developmentally delayed girl with mild dysmorphic features and sideroblastic anaemia

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History: 5 year old girl (no sibs) of non-consanguineous parents with psychomotor developmental delay (IQ ~80), sideroblastic anaemia, microcephaly (<- 2,5 SD) and short stature (4 cm < -3 SD), hypotonia, exercise intolerance, myopia, immunological problems an recurrent infections.
Clinical examination showed mild facial dysmorfisms with an upslant of palpebral fissures, hypertelorism (mum too), hypoplastic alae nasi, a smooth philtrum, small chin, and cup-shaped and low implanted ears. Furthermore, there was a tapering of the fingers, clinodactyly of the fifth digits, vitiligo and eczema.
Biochemical and Molecular investigations: a slightly elevated lactate was shown in blood with no amnio acid abnormalities and a (probably secondary) growth hormone deficiency. There were no abnormalities in karyotyping (46,XX); microarray CGH analysis, Mitomycin C (Fanconi anaemia), mtDNA (including Pearson), RPS19 and RPS14 (Blackfan Diamond), SHOX, FGFR3, NBN (Nijmegen Immunodeficiency syndrome) and several sideroblastic anaemia genes (SLC25A38; GLR5, STEAP3, ABCB7, ALAS2. NGS (WES) identified homozygosity for: 1) a pathogenic nonsense mutation in PUS1 which is associated mitochondrial myopathy with lactic acidosis and ringed sideroblasts (MLASA); and a pathogenic splice site mutation in 2) PFKM, which is associated with Glycogen Storage disease VII (Tarui disease). Her clinical phenotype is predominantly explained by MLASA, but both disorders fit with her clinical problems. The father was a heterozygous carrier, but the mother was not.
Conclusion: This girls' phenotype was explained by an extraordinary genotype. She had (at least) two rare recessive disorders based on a proven paternal isodisomy of chromosome 12 resulting in homozygous pathogenic mutations in PUS1 and PFKM.

PM11.130

Velocardiofacial Syndrome: Review of the Five Years Experience

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Introduction: Chromosome 22q11.2 deletion syndrome, also known as velocardiofacial syndrome / DiGeorge syndrome is one of the most common microdeletion syndromes . Its incidence is 1/4000 in live births. Common manifestations are conotruncal heart defects, palatal abnormalities, hypocalcemia, immune deficiency, velopharyngeal incompetence and intellectual /behavioral problems. We aim to document the most common features of our patients diagnosed during the antenatal period and childhood.
Materials and Methods: A total of 27 cases diagnosed at our hospital between years 2010-2014 were analyzed retrospectively. The variables analyzed were sex, age at diagnosis, presenting manifestation, type of heart defect, other features and positive family history.
Results: From total of 24 children 15 (63 %) were female, 9 (37 %) were male, 3 were antenatal cases. The youngest patients were 1 month old (8), 16 were younger than 1 year, the oldest was 11 years old. The prenatal cases were diagnosed between 25th and 32nd weeks of gestation. Heart defects were the most common presenting manifestation (23/27). Hypocalcemia, immune deficiency, velopharyngeal incompetence and dysmorphic features were the other features in the rest (4/27). The most common cardiac defect was co-existence of VSD and ASD (6), the others were Tetralogy of Fallot (4), truncus arteriosus (4), interrupted aortic arch (3) and VSD (3). Families were tested in 10 cases; 4 of them were found affected.
Conclusions: In the light of these results, we planned to develop a guideline for the diagnosis of velocardiofacial syndrome in high risk pregnancies and newborns in our clinic.

PS11.131

A homozygous PAX3 mutation leading to severe presentation of Waardenburg syndrome with prenatal diagnosis

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Waardenburg syndrome (WS) is one of the most common forms of syndromic deafness, characterized by the clinical association with pigmentation abnormalities of the eye, skin and hair. Four subtypes are described (WS1-4), type 1 and 2 being the most frequent and 3 the rarest. WS1 is caused by heterozygous mutations in PAX3, a transcription factor playing a key role in neural crest cells and muscle development. More than one hundred mutations have been published. Two homozygous mutations in PAX3, leading to hindlimb defects (WS3), were also described.
Here we document 2 consecutive severe cases of WS with fetal presentation. Ultrasound found increased nuchal translucency, lack of active movements, club hands and feet, and neural abnormalities. Both pregnancies were medically interrupted due to the severity of the phenotype.We identified in both fetuses a homozygous missense mutation within PAX3 homeodomain, shedding light on the molecular basis of this very rare form of WS. The consanguineous parents are heterozygous for the mutation and present with a classical form of WS1.
In vitro experiments were carried out to confirm the deleterious effect of this mutation. We found that PAX3 activation ability on the MITF promoter, alone or together with SOX10, was crippled by the mutation. While the cellular localization of the protein was not affected, the mutation clearly interferes with the trans-acting activity of PAX3 and thus inhibits it from activating downstream targets in the neural crest pathway.

PM11.132

Exome sequencing of patients with Weaver-like features links another cancer gene to overgrowth syndromes

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Our lab was the first to publish that constitutional mutations in the epigenetic regulator *EZH2* cause Weaver Syndrome (WS). WS is characterized by overgrowth, increased height, large head, intellectual disability and susceptibility to various cancers. We found pathogenic mutations in *EZH2* among 7 out of 45 individuals with Weaver-like features, of which two had developed malignancies prior to referral. Our efforts focus on determining characteristics that will help predict the likelihood of WS patients developing cancer, through phenotype/genotype correlations and functional studies.
Given that *EZH2* is a histone-modifying enzyme known to be mutated in various somatic cancers, we hypothesized that constitutional mutations in other epigenetic regulators could explain the overgrowth features seen in our undiagnosed patients. To investigate this, we carried out whole exome sequencing for a subset of patients in our cohort.
In one patient, we identified a novel *de novo* mutation in a key epigenetic regulator. Based on the similarities of the patient's phenotype to WS and other

lines of evidence including animal models, we characterized this mutation as probably pathogenic. This is the first report of overgrowth associated with a constitutional mutation in this gene, which is mutated somatically in various cancers. Further investigations are needed to make a definitive link between mutations in this epigenetic regulator, Weaver-like syndromes, and cancer development. We intend to present the identity of this gene and results of further studies at this meeting.

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PS11.133

Heterozygosity of ELN can cause tortuous cerebral artery in Williams syndome

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Introduction: Williams syndrome (WS) is a contiguous gene syndrome commonly caused by a 1.5-Mb deletion involving ELN located at 7q11.23. Elastin arteriopathy plays a key role in WS. Although cardiovascular diseases such as supravalvar aortic stenosis and peripheral pulmonic stenosis are the most significant features, there were some reports of cerebral vessel stenoses, which cause cerebral infarction or moyamoya disease. Neurovascular abnormalities have still not fully been studied in patients with WS.
Materials and Methods: In this study, we investigated magnetic resonance imaging and angiography findings of the brain and intracranial vessels in WS. Four patients were included in this study. Three were typical WS (age range, 1-2 years) diagnosed by fluorescence in situ hybridisation, and the fourth was a 7-year-old girl with an interstitial 7.92Mb deletion of 7q11.21-q11.23 containing the whole ELN gene and a part of the LIMK1 gene. She had a history of peripheral pulmonary stenosis, rectal prolapse, and skeletal features similar to those seen in WS.
Results: Bilateral tortuosity of middle cerebral artery and vertebrobasilar artery were observed in all four cases. Neither constriction of vessels nor infarction was detected.
Conclusions: Tortuosity of the intracranial artery is one of the clinical manifestations in WS. ELN may play an important role in pathogenesis.

PM11.134

Williams-Beuren syndrome: phenotypic, biologic and molecular characteristics

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Introduction: Williams-Beuren syndrome (WBS) is known as a multisystem disorder caused by the deletion of several genes on chromosome 7 (7q11.23). We have used specific facial anomalies, intellectual disability, cardiac abnormalities, and hypercalcemia as diagnostic selection criteria, and used multiple ligation-dependent probe amplification (MLPA - MRC Holland) analysis to confirm the diagnosis.
Materials and Methods: We have analyzed 24 patients confirmed with WBS, and identified 5 cases related to a familial disorder and 19 isolated cases. We have analyzed the clinical and biological characteristics of the above-cited individuals and correlated them with the deletions identified using MLPA P064 kit. Deletion size was further characterized using MLPA follow-up kit P-029-A1.
Results: The data show that 21 of the patients had a hemizygous deletion of ELN, STX1A, FZD9, CYLN2, and LIMK1 genes, whereas three individuals had an atypical deletion (a smaller one that does not extend telometically futher than LIMK1 gene) with an incomplete phenotype (mild dysmorphic face, supravalvular aortic stenosis and learning difficulties). The dysmorphic face was present in all individuals, but the frequency of different features varied. 71% of subjects associated congenital heart defects, whereas hypercalcemia was found only in 19% of the patients with WBS. All subjects had mild to moderate intellectual disability.
Conclusions: For countries with limited financial resources, the use of a combination of MLPA kits allows both confirmation and estimation of the deletion size in WBS patients. A comprehensive phenotype-genotype correlation is provided, with detailed illustration of particular cases.

PS11.135

A partial deletion of the Williams-Beuren syndrome (WBS) region in a child with classic Williams facial features: further delineation of genotype-phenotype correlations in the WBS region

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This patient was referred to clinical genetics following investigations for trisomy 21 and Beckwith-Wiedemann syndrome due to dysmorphic features, mild motor delay and relatively large size. On examination she was noted to have facial features typical of Williams-Beuren syndrome (WBS) and a systolic murmur. Cardiac investigations identified mild left ventricular hypertrophy, mild mitral valve prolapse with regurgitation, and hypertension requiring treatment with amlodipine. Microarray analysis detected an atypical deletion of 1.6Mb at 7q11.23 partially overlapping the WBS region. Imbalance of this region has not previously been reported in the literature.
WBS is a microdeletion syndrome caused by a recurrent deletion of 1.5-1.8 Mb at 7q11.23. The syndrome is characterised by a specific phenotype that includes cardiac, facial, cognitive, endocrine, growth, and connective tissue features. The microdeletion typically encompasses 26 to 28 genes. While deletion of ELN is established as responsible for the cardiovascular phenotype, the exact phenotypic impact of the other genes in the region is not yet clear. Overlapping deletions provide an important insight into genotype-phenotype correlations. It has been suggested that BAZ1B is a candidate for craniofacial development and thus the facial features of WBS. However, our patient has typical facial features and preservation of BAZ1B. Our patient's atypical feature of height velocity along the upper centiles is also of great interest. Here we present a review of atypical deletions reported in the literature, including our new case, and summarise the current knowledge of genotype-phenotype correlations in WBS.

PM11.136

Genotype-phenotype correlation in three patients with Wolf-Hirschhorn syndrome

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Wolf-Hirschhorn syndrome (WHS) (OMIM 194190) is a multiple congenital anomalies/mental retardation (MCA/MR) syndrome caused by partial 4p deletion that was first described independently by Wolf et al. and by Hirschhorn et al. in 1965.
Clinical signs and symptpms include typical facial appearance, resembling the ''Greek warrior helmet'' profile, mental retardation, severe growth delay, hypotonia, congenital heart malformations, midline defects, such as cleft palate and hypospadias, ocular colobomas, renal abnormalities and seizures. Different phenotypes and clinical findings are caused by the number of genes deleted on the 4p16.3 locus.
Here we represent the clinical findings of three patients who have deletion on 4p16.3 chromosomal locus. Size of the deletions were different from each other.
The first patient is a 3,5- year-old female who has afebrile convulsion, hearing loss and speech delay. Submicroscobic heterozygous deletion including PIGG, GAK, FGFRL1, SPON2 genes on 4p16.3 was determined by MLPA method performed with P373-A1 MLPA probemix.
The second patient is a 2-day-old female patient. She has a dysmorphic face appearence, preauricular pit, PDA? and sacral dimple. She has a 46,XX,del(4)(p15.?3) karyotype. A 19 Mb heterozygous deletion between 4p16.3-4p15.3 regions was determined by arrayCGH [CytoScan750K_Array, Affymetrix] method. Deleted region includes 88 genes described in OMIM.
Third patient is a 9-year-old male who has microcephaly, dysmorphic face appearence, iris coloboma, exophtalmus, ASD+PDA+ pulmonary stenosis, gastroesophageal reflux, scoliosis and seizures. He has a 46,XY, der(4)t(4;18)(p16.3;p11.2) karyotype.
Differential expressivity and incomplete penetrance in WHS cause difficulties in clinical diagnosis and problems in understanding the genotype/phenotype correlations.

PS11.137

Cytogenetic approach of girls with short stature: unrelated 6 cases with structural X chromosome abnormalities

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There are various genetic causes of short stature. It has been known that short stature is associated with several disorders including wide variations

of chromosomal abnormalities. A detailed clinical evaluation is essential to suspect and diagnose the underlying cause, especially in patients with overlapping phenotypes.
This work presents the cytogenetics results found in Romanian female patients with short stature as a main clinical feature for referral to Genetics Department. G-banding and metaphase FISH techniques were performed and showed various X chromosome abnormalities: Xp and Xq deletions [46,X,del(X)(p11.2), 46,X,del(X)(q22)], isochromosome X [mos 45,X/46,X,i(X)(q10)], 46,X,idic(X)(p22.1)], ring chromosomes [mos 45,X/46,X,r(X)].
Phenotypic expression of Turner syndrome patients is largely dependent on the patient's karyotype and is most commonly associated with a 45,X karyotype, with a wide spectrum of clinical features. In some patients with Turner syndrome the karyotype shows mosaicism, including cell lines with monosomy X along with X structural anomalies, with possible imbalance of gene content of the X chromosome. A ring X chromosome with loss of XIST gene function and certain X-autosomes translocations are the only sex chromosome structural abnormalities likely to cause mental retardation. Haploinsufficiency of SHOX gene, located on the short arm of the X chromosome, is responsible for height deficit in Turner syndrome patients.
Genetic evaluation of X chromosome rearrangements is important for an accurate diagnosis and to provide information regarding natural history, prognosis, available treatment, recurrence risk.
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PM11.138
Mutations in KCNH1 and ATP6V1B2 cause Zimmermann-Laband syndrome

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Zimmermann-Laband syndrome (ZLS) is a developmental disorder characterized by facial dysmorphism with early onset gingival enlargement, intellectual disability, hypo/aplasia of nails and terminal phalanges of hands and feet, and hypertrichosis. We report that heterozygous missense mutations in KCNH1 account for a significant proportion of ZLS. KCNH1 encodes Eag1/Kv10.1, a member of the ether-à-go-go family of voltage-gated K+ channels. Patch-clamp recordings in cells expressing homomeric KCNH1 mutant channels revealed a shift in the threshold for K+ current activation to more negative potentials for all but one mutant. For the latter, co-expression experiments demonstrated a dominant action over the wild-type channel, with reduced K+ conductance of the heterotetrameric channels at depolarizing potentials but a pronounced conductance at negative potentials. These data support a gain-of-function effect of all KCNH1 mutants. We also report that a single missense change in ATP6V1B2 underlie a small fraction of ZLS. ATP6V1B2 encodes the B2 subunit of the vacuolar H+-ATPase, a multimeric enzyme that mediates acidification in organelles by pumping protons against an electrochemical gradient. Structural analysis indicated a possible perturbing effect of the introduced residue on complex assembly. Our findings provide evidence that disturbances in voltage-dependent K+ currents cause the clinically recognizable ZLS phenotype, and document genetic heterogeneity for this disorder.

PS12.001
Detection of extensive driver mutation heterogeneity in 5 Bulgarian patients with adenosquamous carcinoma of the lung

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Background: Lung cancer remains amongst the leading causes of death worldwide. Adenosquamous carcinoma of the lung is a relatively rare subtype of non-small cell lung cancer accounting for approximately 1-4% of all cases. The genetic mechanisms underlying the onset and progression of this type of cancer are still not clear. The aim of this study was to gain an insight into the mutational profile of tumour tissue from 5 Bulgarian patients with adenosquamous carcinoma of the lung.
Materials and Methods: DNA was extracted from formalin-fixed, paraffin-embedded tumour tissue. Libraries were prepared for sequencing using a TruSight Cancer Panel and included the exons of 94 cancer genes and 284 SNPs associated with cancer. Sequencing was performed on an Illumina MiSeq platform. The BWA pipeline was used for alignment and variant calling.
Results: In each of the patients we identified at least one clear-cut driver mutation leading to a loss of function of a haploinsufficient gene. The genes affected were *APC* (p.Ser2497Ter; p.Gly857Ter; c.730-1G>T), *TP53* (p.Arg196Ter; p.Val197Met), *BRCA2* (c.9089_9090insA), *ATM* (p.Tyr313Ter) and *NF1* (p.Arg304Ter). Each patient also carried other somatic and germline potential driver mutations such as heterozygous nonsense mutations in the haplosufficient FANC family genes (*FANCA*, *FANCC*, *FANCG*, *FANCI*, *FANCM*), as well as many novel missense mutations in other genes predicted as pathogenic by the RadialSVM algorithm.
Conclusion: We observed great heterogeneity in all patients, which highlights the need for a personalised approach in designing a therapeutic strategy for lung cancer patients in order to maximise their chances for successful treatment.

PM12.002
Next-generation sequencing as a tool for assessing minimal residual disease in AML patients with CEBPA mutations

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Introduction: Mutations in the CCAAT/enhancer binding protein alpha (*CEBPA*) gene occur approximately in 5 – 10 % of acute myeloid leukemia (AML) patients and can be used as molecular markers for monitoring of minimal residual disease (MRD). However, detection of MRD using quantitative real-time PCR (RT-qPCR) is complicated due to GC-rich regions and thus validation of leukemia-specific and sensitive MRD assay can be technically difficult. The goal of our work was the application of next-generation amplicon-based deep sequencing (NGS) as a quantitative detection method for MRD monitoring.
Materials and methods: Since 2010, we have performed mutational analysis of the *CEBPA* gene coding region in 411 AML patients at initial diagnosis using Sanger sequencing. In patients with the *CEBPA* mutation as the only detected mutation, we designed leukemia-specific assay using RT-qPCR. However, in 5 patients the assay did not provide sufficient sensitive and specific detection of residual leukemic cells. In these cases MRD was monitored by NGS technology.
Results: From January 2013 we examined 38 samples from 5 AML patients. The assay detection sensitivity achieved the threshold of 10^{-4} to 10^{-5} (1 leukemic cell in 10 000 to 1 leukemic cell in 100 000 cells). Dynamics of residual leukemic cells correlated with clinical outcome of the patients. In patients with relapse, the occurrence of the *CEBPA* mutation was also confirmed by conventional Sanger sequencing.
Conclusion: Quantitative assessment of *CEBPA* mutations using NGS offers a promising way for the detection of MRD level necessary for individualized monitoring of disease status and treatment efficacy.

PS12.003
Polymorphisms of DNA repair genes in the susceptibility of acute myeloid leukemia (AML)

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Alterations in the polymorphisms of DNA repair genes have been associated with the pathogenesis of leukemia. DNA double-strand break repair pathway represents the main pathway in maintaining genome stability which is distinguished into two pathways: homologous recombination (HR) and non-homologous end-joining (NHEJ). The Rad51 proteins are essential components of the HR, whereas Lig4 proteins represent central components of the NHEJ. In this study, we investigated possible implications of the G135C and C26T germline polymorphisms of RAD51 and LIG4 genes, respectively, in AML development and its specific chromosomal abnormalities. Genotyping was performed in 83 patients and 91 controls by PCR-RFLPs. The polymorphic site G135C of RAD51 gene showed the same allelic and genotypic frequency between patients and controls. The genotypic distribution of

C26T polymorphism of LIG4 gene revealed a statistically higher frequency of the variant genotypes in patients compared to controls (C/T: 50.6% vs 30.8%, T/T: 10.8% vs 6.6%, respectively, p=0.006). Allele frequency distribution analysis for LIG4 gene, showed that patients exhibited an almost 2-fold increased risk of carrying at least one mutant allele (T) compared to controls (p=0.004). No statistically significant associations were found for both polymorphisms after stratification of patients according to karyotypic findings. However, an increased frequency of variant genotypes of C26T polymorphism of LIG4 gene was observed in patients with -7/del(7q), -5/del(5q) and +8, compared to controls. Our results showed that the AML risk was not associated with RAD51 gene polymorphism; however, our data provide evidence for an important role of the C26T polymorphic site of the LIG4 gene in AML development.

PM12.004

Principal clinical features of acute myeloid leukemia with mutations DNMT3A R882

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The aim of the research was to analyse frequency of DNMT3A mutations in AML patients, their association with clinico-hematologic parameters and prognostic significance. The investigation group included 143 AML patients. Mutations DNMT3A R882 were identified in 23 (16,1%) patients: R882H - 16, R882C - 6, R882S - 1.
Patients with DNMT3A R882 had higher WBC (p=0,001) and platelets (p=0,020) count at diagnosis and more frequently belonged to FAB groups M5 (p=0,003) and M4 (p=0,012), as compared with DNMT3Awt. Of 23 patients who had AML with DNMT3A mutations, 17 (24,3%) had tumors with normal cytogenetic profiles (of a total of 70 cytogenetically normal samples) (p=0,009). Patients with isolated DNMT3A mutations were seen in 4 cases, whereas in the rest of patients they were detected simultaneously with mutations in genes FLT3, NPM1, NRAS and CKIT. DNTM3A mutations were significantly more prevalent in NPM1mut (p=0,005) and FLT3-ITD (p=0,005) positive cases than wild type. DNMT3A mutations associated with negative influence on patients overall survival (OS) and risk of relapse, compared with DNMT3Awt (Me of OS and RFS: 5,2 and 13,0; 4,8 and 10,0 months; p = 0,031 and p = 0,045, respectively).
Summary. AML with DNMT3A mutations represent the group, homogeneous on a number of clinical and laboratory parameters. DNMT3A mutations are highly recurrent in patients with de novo AML with an intermediate-risk cytogenetic profile. The presence of DNMT3A mutations can be considered as an independent adverse prognostic factor for survival, suggesting that testing of DNMT3A mutations can help further improve risk stratification in AML patients.

PS12.005

Bioinformatics analysis of mature mi-RNA motifs distribution in tumor suppressor genes surroundings

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Every human tumor type has its own unique mi-RNA expression profile. MiRNA can be located in intergenic spaces, in antisense strain, in introns and exons. Such genome organization can determine the mechanism of coordination between RNA and protein expression, but also represents the value of mi-RNA motifs in human evolution. Based on the findings that genome functioning is connected with its structure we have conducted the bioinformatics analysis of mature mi-RNA motifs distribution in tumor suppressor genes surroundings.
We analyzed the intergene spaces located in surroundings of the tumor suppressor genes (APC; BRCA1; BRCA2; CDKN2A; DCC; MEN1; NF1; NF2; PTEN; RB1; TP53; VHL; WT1). Sequences were obtained from NCBI data base and miRBase release 21 using E-utilities API. Motif search was carried out with MEME Suite program package. The results were filtered to yield only those matches with 85% identical nucleotides.
The entire set of non-coding DNA sequences contained 755 motifs of 19-23 nucleotides, homologous to 261 mature mi-RNA sequences. About 60% of all motifs were homologous to miR-5585, miR-1273g, miR-619, miR-5196, miR-5095, miR-709 and miR-1285. These motifs can be considered as non-specific and widely spread in human genome. We have found that tumor suppressor genes have specific patterns of mi-RNA homologous motifs distribution. Prevalent motif type and the density of motif distribution varied from gene to gene. Results can be discussed as a background to the search of new targets for tumor diagnostics and therapy.

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PM12.006

Polyposis coli due to low APC somatic mosaicism

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PURPOSE: To present a patient with familial adenomatous polyposis (FAP) caused by adenomatous polyposis coli (APC) somatic mosaicism; Description: we report of a twenty-one year old female presented with rectal bleeding and abdominal pain. Colonoscopy and esophagogastroduodenoscopy revealed extensive polyposis of the recto-sigmoid junction, distal sigmoid, proximal right colon and cecum. The rectum was essentially spared aside from two small pedunculated polyps. The stomach and duodenum, including the papilla, were normal. In preparation for recto-sigmoid sparing surgery, more than sixty polyps were removed. The patient had no extracolonic signs of FAP. Her maternal grandmother was diagnosed with colon cancer at age seventy-six, but there was no other family history of polyps or colon cancer. Methodology: Next-generation sequencing (NGS) analysis was performed using the ColoSeq™ panel* on DNA extracted from both peripheral blood lymphocytes and colonic polyps. RESULTS: Molecular analysis detected the p.E1408X deleterious mutation in the APC gene in 12 of 276 (4%) reads of the DNA in the peripheral blood and in 30% of the DNA from colonic polyps. CONCLUSIONS: In this patient, 4% APC mosaicism of the peripheral blood lead to florid polyposis. Somatic mosaicism has been reported to cause cancer syndromes in a few cases, but has been underestimated. This case should reinforce the need for NGS analysis in all patients with a personal history of polyposis, no family history of colon polyps/cancer, and no identified germline mutation by traditional less sensitive approaches.

PS12.007

Analysis of BCR-ABL mutations in chronic myeloid leukemia patients treated with tyrosine kinase inhibitors

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Mutations in the BCR-ABL tirozin-kinase (TK) domain represent the most common mechanism of resistance to personalized therapy with TK inhibitors (TKI) in patients with chronic myeloid leukemia (CML).
Mutational status of the BCR-ABL gene corresponding to the TK domain was analyzed by capillary sequencing in 45 CML patients with suboptimal response/failure to TKI, in order to tailor their therapy. The response of the patients to TKI therapy was monitored at molecular and cytogenetic level.
Mutations in the BCR-ABL gene were identified in 18 (40%) patients: a single mutation in 15 patients and 2 mutations in 3 patients. Mutations identified corresponded to several regions of the BCR-ABL oncoprotein, such as: the P-loop (M244V, G250E, Q252H), the ATP-binding region (L298V, V299L, T315I, F317L), the SH2-contact region (M351T) and the substrate-binding region (F359V). The T315I mutation, conferring resistance to almost all known TKI, was detected both as single mutation (in 7 patients) and in combination with M351T (1 patient).
The 2 mutations detected in the BCR-ABL TK domain in case of 2 patients represented different clones; during dasatinib therapy, the resistant clones were selected (M351T and T315 respective), while the clones sensitive to this drug have disappeared.
Different chromosomal abnormalities associated with clonal evolution were identified in 3 patients, which may be the major cause of secondary resistance to TKI.
Conclusions:
- Mutational status of the BCR-ABL TK domain is valuable information for the best therapeutic decision and management of patients with CML;
- Additional TKI resistance mechanisms can be detected by a combined molecular and cytogenetic monitoring of the CML patients.

PM12.008

Changes in the gene expression and copy number aberrations in non-invasive and muscle-invasive bladder tumors

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Background:
The aim of this study was evaluation and comparison of the copy number aberration and the expression of the genes, related to cancer drug resistance in non-invasive and muscle invasive bladder carcinomas.
Materials & Methods:
Bladder transitorial cell cancer samples from pTa, pT1, pT2, pT2a and pT2b stages were tested for gene expression levels and copy number aberrations. A gene expression analysis of the 84 genes from Cancer drug resistance and metabolism panel (Qiagene) of 30 tumours and CytoChip Oligo aCGH, 44K format (Bluegnome) of 12 tumours were performed.
Results:
The DNA copy number data shows gains, amplifications and loses in the bladder cancer genome, compared to the control. Tumours from pT2, pT2a and pT2b stages have higher numbers of chromosomal imbalances then non-invasive tumours.
The gene expression analysis of the bladder tumours revealed an up-regulation for CYP1A1, CYP3A5, AR, CLPTM1L, CCNE1, MVP, TOP2B, AHR and PPARG genes compared to the normal tissue. A statistically significant difference (p<0,0001) in the expression level in muscle invasive versus non-invasive bladder tumours of the EGFR, ERBB2, ERBB4, ABCC1, ABCC3, ARNT, CYP1A1, CYP3A5, EPHX1, MVP and PPARG genes is observed. These genes are involved in the multi-drug resistance and the metabolism of: steroid hormones, cyclosporine, polycyclic aromatic hydrocarbons, and anticancer drugs Vincristine, Thiopurine, and Taxol. This data confirms the significance of these genes as targets for further clinical trials.
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PS12.009

Study of tumour recurence in superficial transitional cell carcinoma by microarrays

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Treatment of bladder superficial tumours is dependent on the risk of recurrence and it is therefore clinically important to identify bladder cancers with a high risk of intravesical recurrence after transurethral bladder tumour resection. For the improvement of recurrence prognosis we applied gene expression microarray analysis to two groups of bladder tumours (superficial bladder tumours with no or late recurrence during the period of two years versus early recurrence ones). Data from microarrays containing 29,019 targets (Applied Biosystems) were subjected to a panel of statistical analyses to identify bladder cancer recurrence-associated gene expression changes. After validation 33 genes manifested significant differences between both groups. The significant expression was observed in the group of patients without recurrence by 30 genes of which the highest differences were detected by NINJ1, GNE, ANXA1, TNFSF15, WDR34, ARHGEF4, PRICKLE1, PSAT1, RNASE1, TM4SF1, TSPAN1, PLOD2 and WDR72. These genes code for signal transduction, vascular remodeling and vascular endothelial growth inhibition mainly. Specially, PRICKLE1 and TNFSF15 genes were described to be linked with WNT/β-catenin signaling and angiogenesis regulation and MTOR pathway. Loci of genes with significant changes of gene expression were on characteristic chromosomes for bladder cancer: 9q, 17q, 2q and 16p. On the basis of these findings we documented a number of expression changes of genes among which some seem to form clinically useful recurrence markers of superficial bladder tumours. Research was supported by MSM 0021620808 and Diana Lucina.

PM12.010

Routine and emergency BRCA1 and BRCA2 genetic testing using MASTRDx (Multiplicom®) kit, and MiSeq (Illumina®) sequencer: Description of two NGS-Workflows.

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Background
BRCA1 and BRCA2 are major genes involved in hereditary breast and ovarian cancer (HBOC). Facing an increasing number of routine genetic tests and emergency indications such as eligibility to targeted therapies (anti-PARP) and surgery decision, we optimized our genetic testing strategy to reduce processing time. Based on high-throughput sequencing, our approach allowed the bi-monthly screening of 96 patients in routine diagnosis (RD), and the additional testing of 16 patients every three weeks as part of a fast track (FT) was performed.
Methods
96 DNA samples (RD) or 16 DNA samples (FT) were amplified using BRCA MASTRDx kit (Multiplicom®), based on five multiplex PCR covering coding regions and exon-intron junctions of BRCA1 and BRCA2 genes, followed by sequencing on MiSeq (Illumina®). Bioinformatic analysis was performed using SeqNext Software (JSI®). Large rearrangement were researched using MLPA (MRC Holland®).
Results
1154 patients were analysed in 2014 (1070 RD and 84 EI): 10.3% of them had germline deleterious mutations (CNV included), and 6.9% carried variants of unknown significance. The Next-Generation Sequencing (NGS)-based strategy amorced the shortening of analysis delay, which get closer to our target delay of 16 weeks maximum. The fast track process, including medical validation, last less than 8 weeks.
Conclusion
This NGS approach enabled to increase the throughput of genetic tests for BRCA1 and BRCA2, and to develop an efficient fast track in response to therapeutic emergency indications. The use of two MiSeq sequencer enables a technical capacity of 2500 patients a year. Furthermore, this technological resource allows us to consider HBOC genes panel sequencing.

PS12.011

Routine germline BRCA testing in serous ovarian cancer: The West of Scotland experience

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Background: Germline mutations in BRCA1/2 (gBRCA) are strongly linked to non-mucinous ovarian cancer (OC), in particular high grade serous OC (HGSOC). Since Jul 13, all patients with non-mucinous OC in the West of Scotland (WoS) have been offered germline BRCA1/2 testing regardless of family history. All patients received counselling prior to testing. Methods: Sequencing results were collated with clinical data. Progression-free (PFS) and overall (OS) survivals in gBRCA mutation carriers and non-carriers were compared using Gehan-Breslow-Wilcoxon test. Results: 154 patients were referred to genetics and 120 accepted testing. gBRCA mutations were identified in 22/120 (18.3%) - 6 BRCA1, 16 BRCA2. An additional 4/120 (3.3%) had variants of unknown significance (VUS). In HGSOC, the mutation rate was 21/92 (22.8%) (6 BRCA1, 15 BRCA2). 50% mutations occurred in women with Manchester score (MS) ≤ 15: these would have been missed if family history alone determined decision to test. OS for gBRCA2 carriers with stage III/IV HGSOC was significantly better than gBRCA wild-type (HR: 2.25 95%CI 1.05-4.79, p = 0.02, gBRCA wild-type median 63.2m, BRCA2 carriers median undefined). Median PFS of gBRCA2 carriers was also significantly better than gBRCA wild-type (27.9m vs 15.1m, HR 1.8, 95%CI 1.08-3.26 p = 0.02). For gBRCA1carriers both PFS and OS did not differ significantly from non-carriers. Conclusions: Routine germline BRCA1/2 mutation testing is acceptable and feasible in women with OC. c.20% women with HGSOC carry a gBRCA mutation. In WoS, we observe higher prevalence of BRCA2 mutations (16.3%) than previously reported (6-8%). In our cohort, gBRCA2 mutation confers better PFS and OS than wild-type BRCA1/BRCA2 status.

PM12.012

BRCA1/2 Mutation Status Is an Independent Factor of Improved Survival for Advanced Stage Ovarian Cancer

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The aim of this study was to evaluate BRCA1 and BRCA2 mutation impact on prognosis of advanced-stage (III-IV) ovarian cancer patients after standard treatment.
Methods: A total of 466 patients with advanced-stage (primary) epithelial ovarian cancer (EOC) were identified from a clinical database during year

1998-2013 and enrolled in a prospective, single-center study. All cases with available germline DNA (n=297) were screened for BRCA1 and BRCA2 gene mutations using combination of methods (HRM, Sanger/Next Generation Sequencing, MLPA). Progression-free survival (PFS) and overall survival (OS) was assessed between BRCA1/2 mutation carriers and BRCA1/2 wild-type patients. To eliminate survivorship bias, analysis was limited for cases with BRCA testing performed <36 month from the diagnosis. Various clinical risk factors for PFS and OS were assessed by univariate and multivariate Cox regression analysis with stepwise model selection process.
Results: Older age (hazard ratio [HR], 1.032; 95% confidence interval [CI], 1.010-1.055; P=0.0047), nonoptimal cytoreduction (HR, 3.170; 95% CI, 1.986-5.060; P=0.0001), and BRCA1/2 wild type (HR, 1.625 [1.003-2.632]; P=0.0486) were significantly associated with shorter PFS in multivariate Cox regression analysis. Nonoptimal cytoreduction (HR, 2.684; 95% CI, 1.264-5.701; P=0.0102) and BRCA1/2 wild type (HR = 1,612 (95% CI 1,16 - 2,23; P=0.0002) were statistically significant risk factors for shorter OS. The overall 5-year survival for the hereditary case patients was better than that of the nonhereditary patients, however after that time no survival advantage was apparent.
Conclusions: Advanced ovarian cancer patients harboring BRCA1/2 mutation treated with debulking surgery and platinum-based adjuvant chemotherapy have a longer PFS and OS not longer than 5-years.

PS12.013

Laying the groundwork for a global solution to the variome: the BRCA Challenge

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Most people at risk of hereditary cancer remain unrecognized. Cost and complexity of interpreting sequenced data are significant barriers. As technical advances reduce the cost of sequencing, and new interventions offer real hope of cancer cure and prevention, the problem of variant interpretation has become more visible.
The BRCA Challenge calls on clinicians, clinical laboratories and researchers across the world to share their knowledge of variation in the BRCA 1 and 2 sequences. Existing databases; BIC, UMD, LOVD, ClinVar, HGMD are being linked to ensure a single point of access. The goal is to provide the community with a reliable record of those variants that can be interpreted as pathogenic for a high penetrance phenotype versus benign. An international interpretation community will include the existing ENIGMA project, which establishes clinical significance of variants in these genes. Meanwhile, an API suitable for the extraction of all relevant sequence variation is being developed, including co-occurrence data, from genomic datasets to better define non-pathogenic variants across population diversity.
Ultimately the database will facilitate exploration of the clinical significance of variants across pathogenicity, building on and contributing to the work of CIMBA (the Consortium of Investigators of Modifiers of BRCA1/2), focused on refining the estimates for penetrance of a subset of variants, as well as ClinGen (Clinical Genome Resource) which provides curated genomic knowledge to improve clinical care. Barriers to be overcome include concerns around the quality of phenotypic data, the reliability of sequences, confidentiality, the standardisation of data collection, and the attribution of credit to generate, collect the data and curate their collection.

PM12.014

Two new cases of double heterozygosity for BRCA1 and BRCA2 gene mutations detected during routine diagnostic screening at the SW Thames Regional Genetics Laboratory

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Introduction: *BRCA1* and *BRCA2* are the two major genes associated with inherited breast and ovarian cancer. Double heterozygosity (DH) is an extremely rare event in which both *BRCA1* and *BRCA2* are mutated simultaneously in a patient. To date, most cases of DH reported are in Ashkenazi populations, but only a few cases have been reported to have more than one non-Ashkenazi BRCA mutation. Here, we have described two families with two different mutations, one in *BRCA1* and another in *BRCA2*. One case includes an Ashkenazi mutation and a non-Ashkenazi mutation while the other case includes two non-Ashkenazi mutations.
Method: MLPA and bidirectional Sanger sequencing analysis of the entire coding region of *BRCA1* and *BRCA2* were carried out.
Result:
Family 1. Family with early onset of breast cancer, our patient was diagnosed at the age of 33 and her mother at the age of 36. In the index case, the c.5266dupC frameshift mutation in *BRCA1* (common Ashkenazi mutation) and the c.9097_9098insT frameshift mutation in *BRCA2* were detected.
Family 2. The proband was diagnosed with breast cancer at the age of 36 and has a family history of breast cancer from the maternal side. Sequencing analysis showed that the patient was heterozygous for the c.3750delG frameshift mutation in *BRCA1* and the c.4447delA frameshift mutation in *BRCA2*.
Conclusions: These findings highlight that a second mutation could potentially be missed if a screen is not completed once a mutation is detected.

PS12.015

BRCA1/2 risk-reducing bilateral salpingo-oophorectomy audit

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Background: BRCA1/2 carriers have a lifetime risk of ovarian cancer of up to 30-50% and no evidence-based ovarian cancer screening is available. Therefore, most women opt for a risk-reducing bilateral salpingo-oophorectomy (RRBSO). Since 2007 the BRCA family service at Guy's Hospital has advised gynaecological surgeons to follow a protocol for RRBSO in BRCA1/2 carriers as standard practice. This evidence-based protocol, implemented after an audit in 2006, includes removal of fallopian tubes, serial sectioning, peritoneal washings, and HRT recommendations.
Methods: An audit of women who had RRBSO between February 2013 to May 2014 (37 women) was undertaken to measure compliance with this protocol and review the evidence for the protocol. Data was collected from the BRCA family service database and clinical notes were reviewed. Information about post-surgical use of HRT was collected from the patient.
Results: 84% (31 women) were between 40 and 59 years at the time of surgery. Removal of fallopian tubes was evident in 97% (36 women), sectioning was undertaken in 76% (28 women) and peritoneal washings was documented in 51% (19 women). This showed an improvement in compliance with the protocol compared to the 2006 audit (36%, 16% and 8% respectively). No pattern was identified between compliance and hospital where surgery was performed.
Conclusion: Compliance with recommendations has vastly improved since the implementation of the RRBSO protocol. Inconsistencies between and within hospitals highlights the need for further discussion with surgeons in our catchment area. Before disseminating the results widely, literature review and revision of protocol is planned.

PM12.016

An Audit of BRCA1 and BRCA2 genetic testing offered to unaffected individuals as part of the cancer genetics service at Guys Hospital

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Introduction
In keeping with recommendations made by NICE guidelines (2013), the cancer genetic services at Guys started offering genetic testing to unaffected individuals who have a combined BRCA1 and BRCA2 mutation carrier probability of 10% or more. Eligibility was determined by confirming ovarian cancers and cancers in first degree relative. In addition families had to have a Manchester score of 17 or over to be eligible. The aim of this audit was to review the data on unaffected individuals who underwent BRCA1 and BRCA2 testing.
Method
We obtained all diagnostic BRCA1 and BRCA2 results between the period of 30/08/2013 and 01/09/2014. These were separated into affected and unaffected individuals. The unaffected individuals were further separated into mutation, VUS or no mutation and their Manchester score was calculated.

Results
From a total of 359 individuals, a total of 29 unaffected individuals were identified. Of these 29, BRCA mutations were identified in 2 (6.9%), VUS were identified in 7 (24.2%) and 20 (68.9%) were negative for either BRCA1 or BRCA2.
Conclusions
We concluded that there was a 6.9% pick up rate for mutations which is lower than the 10% threshold. However, the small sample size may have contributed to this. The individuals in whom mutations were identified had a very high Manchester score (29 and 37 respectively). Interestingly, a very high number (24.2%) of VUSs were also identified. In light of the findings we may need to review our threshold for offering genetic testing to unaffected individuals based on mutation probability, cost implications and high variant rate.

PS12.017
Analytical validation of a CE marked companion diagnostic tumor test to detect BRCA1 and BRCA2 mutations in ovarian cancer for clinical use

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Background: Somatic and germline mutations in the *BRCA1* and *BRCA2* genes may predict outcomes to ovarian cancer therapies such as platinum and PARP inhibitors. To identify patients that may benefit from these therapies, a companion diagnostic test has been developed to analyze tumor specimens for *BRCA1/2* mutations. The aim of this study is to validate the test's analytical performance.
Methods: Genomic DNA isolated from 42 anonymized ovarian tumor samples underwent full sequence and large rearrangement analysis using next generation sequencing (NGS). The criteria for calls required 99% of bases to have ≥ 100 reads. The reproducibility of this test was evaluated by sequencing 10 samples in triplicate across 6 batches. All samples underwent analysis by an independent laboratory to verify results.
Results: The analytical sensitivity was estimated to be >99.07% (lower bound of 0.95 C.I.), with an analytical specificity >99%. The average read depth was approximately 425X per base, with a minimum inclusion criterion of 50X per base. All samples that were previously identified by alternative methods as positive for deletions/duplications were correctly identified using NGS dosage analysis. This study also showed that the results were 100% concordant with independent laboratory analysis - both finding 319 sequence variants - and had 100% intra- and inter-run reproducibility.
Conclusions: Ovarian tumor testing for *BRCA1/2* mutations including sequence and large rearrangement variants using NGS has been validated with high sensitivity and specificity for companion diagnostic use. It may be used for identifying patients with somatic or germline mutations to help guide therapy decisions.

PM12.018
A significant proportion of Greek TNBC patients carry rare loss-of-function mutations in 15 breast cancer predisposing genes

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Triple-negative breast cancer (TNBC) is an aggressive breast cancer subtype which is generally characterized by poor overall survival, mainly attributed to inadequate therapeutic targets. Germline BRCA1 mutations have been strongly associated with TNBC, the prevalence of which can be as high as 29% in populations with strong founder effect. Mutations in other breast cancer predisposing genes can be associated with TNBC, but the contribution of these genes is still unclear.
TNBC patients (n=733; mean age 45.19 years) were recruited solely on their TNBC status, between years 1999-2014. Initially, the Greek founder BRCA1 mutations were tested; individuals who were wild type at these loci were then tested by next generation sequencing using a 27-breast cancer gene panel.
Loss-of-function mutations were identified in 25.3% (186/733) of the individuals tested. BRCA1 mutations were observed in 20.2% (148/733). The mean age of breast cancer diagnosis was 39.6 years. Another 38 deleterious mutations were detected in 14 additional genes. Interestingly, 3.1% of these mutations cluster in only three genes, namely BRCA2 (1.9%), RAD51C (0.68%) and BARD1 (0.54%).
TNBC status alone can be a significant predictive factor for germline mutations in predisposition genes, irrespective of family history and early onset of disease. Even with the application of improved DNA sequencing techniques, BRCA1 loss-of-function mutations are still the major players in TNBC. Mutations in additional genes, which are BRCA1 partners in the homologous recombination pathway, also seem to be overrepresented in TNBC patients. These observations might be beneficial in terms of developing targeted therapeutics, such as poly-ADP-ribose polymerase inhibitors.

PS12.019
Identification of a first large deletion of the BRCA1 gene in a Croatian patient

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Breast cancer is the most common cancer in women after non-melanoma skin cancer, and it is the leading cause of cancer related deaths in Croatia. Ovarian cancer is in the fifth place, both in incidence and mortality. About 5-10% of all breast and/or ovarian cancer cases are hereditary, and heterozygous germline *BRCA1* and *2* mutations are responsible for the majority of hereditary breast and/or ovarian cancers. In the most cases, the mutations are small nucleotide alterations leading to premature stop of translation. Large rearrangements of *BRCA1* gene and less often, *BRCA2* gene have been described in recent years, but haven't been found in Croatia so far.
Here we describe a case of a Croatian breast cancer patient with no apparent family history of cancer, who developed a triple negative breast cancer at the age of 29, with return of the disease at the age of 33. No mutation was found by HRM or sequencing, but the 5-7 exon deletion of the *BRCA1* gene was determined with a Quantitative Multiplex PCR method and confirmed with MLPA analysis.

PM12.020
Whole cDNA analysis of BRCA1 and BRCA2 genes in Spanish breast/ovarian cancer families

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Introduction: Germline pathogenic variants in BRCA1 and BRCA2 genes are associated with inherited high-risk of breast (BC) and ovarian cancer (OC). The whole coding sequence and the flanking exon-intron junctions are routinely screened in genomic DNA. However, in only about 25% of the cases a clear deleterious variant is identified. The disease susceptibility may also be associated with alterations in gene expression due to variants located in splicing and/or transcription regulatory non coding regions, not included in the conventional analysis. To test this hypothesis, we analyze the whole BRCA1/2 cDNA in high-risk Spanish families testing negative for BRCA1/2.
Materials and Methods: Total RNA of 200 probands and 10 control individuals was isolated from peripheral blood leukocytes. PCR overlapping amplicons including the entire cDNA of both genes were generated. Full-length transcripts were qualitatively assessed by QIAXCEL and bidirectional Sanger sequencing, allowing the detection of aberrant transcripts and alterations in natural occurring isoforms. Single polymorphisms in coding regions were also identified to confirm biallelic expression.
Results: To date, one out of 60 patients analyzed presents an aberrant transcript consisting in a partial inclusion of intron 21 in BRCA1. This splicing alteration is yet to be confirmed by capillary electrophoresis of fluorescent amplicons. A potential allelic imbalance of BRCA1 expression has been also detected in another individual.
Conclusions: Although the frequency of splicing and expression alterations in high-risk families appears to be low, the analysis of RNA can provide a more comprehensive BRCA1/2 diagnostic.
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PS12.021
NGS screening for BRCA1/2 in Portuguese high-risk breast/ovarian cancer families: initial results, advantages and challenges

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Introduction: *BRCA1* and *BRCA2* are the genes most frequently involved in hereditary breast/ovarian cancer (HBOC). These genes are very large and the genetic screening based on Sanger sequencing is laborious and time consuming. Last year our Lab acquires a Next Generation Sequencing (NGS) instrument and we change the *BRCA1/2* screening to NGS. **Patients and methods:** Review of all patients screened for *BRCA1/2* by NGS. All patients underwent pre and post-test counselling, were pre-screened for the *BRCA2* Portuguese founder mutation [Machado et al, 2007], analysed for *BRCA1/2* point mutations by NGS with *MASTR-BRCA* assay (*Multiplicom)* and for large rearrangements by MLPA. **Results:** The first stage of NGS implementation included the re-analysis of 11 patients previously positive for a *BRCA1/2* mutation and 100% concordance was obtained for all mutations and variants. Between September 2014 and January 2015, 290 patients were screened and 25 positive patients were detected (8*BRCA1*, 9*BRCA2, 1BRCA1+BRCA2, 2BRCA large* rearrangements, *1CHEK2 and 4* founder mutation), which corresponds to a 8.6% detection rate. **Conclusion:** NGS is a valuable methodology with high throughput and quality, allowing a rapid diagnostic result for more appropriate clinical/prophylactic attitudes. In 5 months, 290 high-risk families were screened and 25 presented deleterious mutations. As expected, several neutral or unknown variants were also detected, posing a significant challenge for counselling. Depending on pedigree reanalysis and multidisciplinary decision, BRCA1/2 negative families will be screened for a selected panel of other relevant genes.

PM12.022

Prostate-Specific Antigen velocity as a predictive biomarker in a prospective prostate cancer screening study of men with genetic predisposition

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Objectives: *BRCA2* mutation carriers are more likely to develop prostate cancer (PrCa) and aggressive disease. We assessed whether men in the IMPACT study (Identification of Men with a genetic predisposition to ProstAte Cancer: Targeted screening in men at higher genetic risk and controls) exhibit altered PSA kinetics prior to diagnosis.
Methods: We calculated PSA Velocity (PSAV) using validated methods: arithmetic mean(a), linear regression(b) and first and last readings(c) equations. Pearson chi-square test was used to compare PSAV between *BRCA1/2* carriers versus negative controls in those that underwent prostate biopsy (PB). Binary Logistic Regression was used to compare PSAV between carriers who were and were not diagnosed with cancer.
Results: PSAV was available for 191 men who underwent a PB; 57 PrCas were diagnosed. There was no difference in the median PSAV between *BRCA1* carriers and non-carriers who were diagnosed with cancer (0.24 vs. 0.37 ng/ml/yr, respectively). In comparison, *BRCA2* carriers with cancer showed a significantly higher PSAV when compared to non-carriers (1.36 vs. 0.50 ng/ml/yr, respectively, p=<0.05). *BRCA2* carriers with a PSAV over 0.75ng/ml/yr were 5 times more likely to be diagnosed with PrCa compared to non-carriers [95%CI=1.5-14; P value=0.003]. In addition, *BRCA2* carriers with cancer were 12 times more likely to have high-grade disease (Gleason score ≥7) phenotype compared to non-carriers with cancer [95%CI: 1.1-98; p=0.039].
Conclusions: This is the first study to document that *BRCA2* carriers have different PSA kinetics compared to non-carriers. This increased PSAV most likely reflects prior associations with disease susceptibility, aggressive disease and PrCa specific mortality.

PS12.023

Uptake of risk-reducing mastectomy in BRCA1 and BRCA2 gene mutation carriers at The Royal Marsden

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Background: Women with a BRCA1 or BRCA2 mutation may choose surveillance with MRI and mammography or risk-reducing mastectomy (RRM) to manage their breast cancer (BC) risk. The reported uptake of RRM in the UK has historically been low, with many women opting for surveillance. However, demand for RRM anecdotally is said to be increasing. Here we report uptake of RRM at the Royal Marsden (RM) Hospital.
Methods: A comprehensive list of female BRCA mutation carriers was obtained from the RM Genetics Unit database. The electronic patient record was interrogated to ascertain decision-making regarding breast surgery.
Results: 858 carriers were identified: 458 with BRCA1 mutations and 400 with BRCA2 mutations. Of the 458 BRCA1 carriers, 82 had therapeutic mastectomy for BC (14 unilateral, 23 bilateral, 45 unilateral with contralateral RRM). Of the remainder 124(33%) chose bilateral RRM and 252(67%) had no breast surgery. Of the 400 BRCA2 carriers, 90 had therapeutic mastectomy for BC (25 unilateral, 21 bilateral, 44 unilateral with contralateral RRM). Of the remainder 76(25%) chose bilateral RRM and 234(75%) had no breast surgery.
Conclusions: Uptake of bilateral RRM in BRCA carriers was slightly higher for BRCA1 vs BRCA2 mutation carriers (33% vs 25%), but this was not statistically significantly different. Rates of contralateral RRM with therapeutic mastectomy for BRCA mutation carriers with unilateral BC were similar for both genes (~50%). Further work is underway to investigate if RRM uptake has been constant or is increasing.
This work was funded by the RM/ICR NIHR BRC.

PM12.024

Emerging phenotype of BRCA2 mutations in South African patients with breast cancer and Fanconi anaemia

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Introduction: Heterozygous mutations in *BRCA2* predispose to Hereditary Breast and Ovarian Cancer syndrome (HBOCS). Further, a rare subtype of Fanconi anaemia (FA) is caused by biallelic mutations in the *BRCA2* (*FANCD1*) gene. Two parallel studies in South African black patients investigating the genetic basis of inherited breast cancer and FA not caused by *FANCG* have identified *BRCA2* as being a potentially important gene.
Subjects and Methods: 84 young black South African women with breast cancer were screened for *BRCA1* and *BRCA2* mutations using either Sanger or NGS sequencing. In a separate study 5 patients with FA were screened for mutations using a NGS panel of known DNA breakage associated genes.
Results: In the women with breast cancer, 9 *BRCA2* and 4 *BRCA1* mutations were identified. Two *BRCA2* mutations observed more than once suggest the possibility of founder mutations. There is no family history in 5 of the women with *BRCA2* mutation. Two had triple negative tumours. An FA patient with multiple birth defects and Wilms tumour had 2 pathogenic truncating *BRCA2* mutations. *BRCA2* sequence variants (unconfirmed pathogenicity) were identified in 2/5 other black patients with clinical diagnoses of FA.
Conclusions: The phenotype of *BRCA2* mutations in South African black patients may be different to that previously described, and needs to be fully characterised. The paucity of family history in young mutation carriers with breast cancer may suggest strong protective environmental effects. Possible overlapping founder *BRCA2* mutations may contribute to both young onset breast cancer and severe FA.

PS12.025

Exome sequencing to explore temporal and spatial mutational evolutionary changes in invasive breast cancer specimens

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Genetic heterogeneity of breast cancer represents a significant challenge with respect to disease course and patient clinical management. Understanding molecular evolution of tumours both temporally and spatially would contribute further insight into molecular derangements driving disease progression. Exploratory analyses have been conducted on exome sequencing data of genomic DNA extracts from six invasive breast carcinoma samples representing matched primary tumour and the corresponding axillary lymph node metastases. Data was mined regarding concordance and discordance between primary tumour samples and their respective metastatic variants. Initial findings suggest that 37 candidate indels are common to all three axillary lymph node samplings and yet absent from the three samplings of the primary tumour. Several genes have been identified as having frameshift mutations caused by indels that may lead to potential abrogation of protein function. Of the genes with indel mutations in their coding sequences include molecular players that have been previously linked to anti-angiogenesis. These initial findings provide the framework for detailed molecular analyses of the molecular evolution of primary breast cancer and their associated metastases.

PM12.026

Panel next-generation sequencing reveals a high prevalence of deleterious ATM mutations in BRCA1/2-negative breast and ovarian cancer families

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Approximately 24% of familial breast cancer (BC) and/or ovarian cancer (OC) cases analyzed within the framework of the German Consortium for Hereditary Breast and Ovarian Cancer (GC-HBOC) are due to pathogenic *BRCA1/2* mutations. However, the mutation frequencies of non-*BRCA1/2* genes associated with familial BC and/or BC/OC are largely unknown. Here, we present the NGS analysis (TruRisk™ gene panel) of a cohort of 574 *BRCA1/2*-negative index cases which comprises 256 unselected patients with triple negative breast cancer (TNBC) and 318 cases from high-risk BC and BC/OC families. By focusing on 21 BC/OC associated genes (*ATM, BARD1, BRIP1, CDH1, CHEK2, FANCM, MLH1, MSH2, MSH6, MRE11A, NBN, PALB2, PMS2, PTEN, RAD50, RAD51C, RAD51D, SMARCA4, STK11, TP53*, *XRCC2*), we identified 40 different pathogenic variants in 38 unrelated mutation carriers derived from 318 high risk BC and BC/OC families (12%). In contrast, only 9 mutation carriers (3.5%) were discovered among the unselected TNBC cases. Interestingly, we identified a high frequency of pathogenic *ATM* mutations *(n=10, 3.1%)* in the familial cases whereas no *ATM* mutations were found in the TNBC cohort. Additionally, we found a high frequency of mutations in *CHEK2*, *PALB2*, *RAD50* and confirm *FANCM* and *SMARCA4* as novel BC/OC predisposing genes. Due to the unexpectedly high mutation frequencies in familial cases, our study highlights the importance of these genes to be included in BC/OC routine diagnostics.

PS12.027

The Estrogen Receptor -α Gene rs1801132 variation and Breast Cancer risk in Iran

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Iranian breast cancer patients are relatively younger than their Western counterparts. Evidence suggests that alterations in estrogen signaling pathways, including ESR1(estrogen receptor-α), occur during breast cancer development in Caucasians. Epidemiologic studies have revealed that age-incidence patterns of breast cancer in Asians differ from those in Caucasians. Genomic data for ESR1in either population is therefore of value in the clinical setting for Iranian breast cancer.
A case-control study was conducted to establish a database of ESR1polymorphisms in Iranian women population in order to compare Western and Asian with Iranian (Asian-Caucasians) distributions and to evaluate ESR1-polymorphism as an indicator of clinical outcome. DNA was extracted from Iranian women with breast cancer referred to Imam Khomeini Hospital Complex clinical breast cancer group (150 patients) and in healthy individuals (147 healthy control individuals). PCR single-strand conformation polymorphism technology was performed.
A site of silent single nucleotide polymorphism (SNP) rs1801132 was found, The frequency of allele 1 in codon 325 (CCC→CCG) was significantly higher in breast cancer patients (39.6%) than in control individuals (28.9%; P = 0.007). The allele CCG had also significant association with the occurrence of lymph node metastasis.
Data suggest that ESR1polymorphisms in exon 4 codon 325 is correlated with various aspects of breast cancer in Iran. ESR1 genotype, as determined during presurgical evaluation, might represent a genetic marker for predicting breast cancer lymph node metastasis.

PM12.028

Nonsense mutation in FANCM confers risk for triple-negative breast cancer

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Inherited predisposition to breast cancer is known to be caused by loss-of-function mutations in *BRCA1, BRCA2, PALB2, CHEK2*, and other genes involved with DNA repair. However, most families severely affected by breast cancer do not harbor mutations in any of these genes.
In Finland, founder mutations have been observed in each of these genes, suggesting that the Finnish population may provide a unique resource for identification of additional breast/ovarian cancer alleles. We studied 24 breast cancer patients from 11 Finnish breast cancer families with exome sequencing and further genotyped selected DNA repair variants in 3166 familial and/or unselected breast cancer patients as well as 569 ovarian cancer patients and 2090 population controls. Of all genotyped variants, a nonsense mutation (c.5101C>T, rs147021911, p.Gln1701Ter) in Fanconi Anemia complementation gene M (*FANCM*) was significantly associated with breast cancer risk (OR = 1.86, 95% CI = 1.26-2.75, *P* = 0.0018). Further assessment based on tumor pathology identified a particularly strong effect in triple-negative breast cancer (OR = 3.56, 95% CI = 1.81-6.98, *P* = 0.0002). These findings identify *FANCM* as a novel breast cancer predisposition gene, with a moderate risk of especially triple negative breast cancer for mutation carriers.
The study has been supported by the Helsinki University Central Hospital Research Fund, the Academy of Finland, the Sigrid Juselius Foundation, Nordic Cancer Union, and the Finnish Cancer Society. FJC was supported by the Breast Cancer Research Foundation, and CA116201 NIH SPORE award in breast cancer to the Mayo Clinic.

PS12.029

Associations Between HER2/neu ,TOP2A ,Chromosome 17 Copy Numbers and TWIST , RARβ2 and ESR1 Gene Promotor Hypermethylations of Patients with Breast Cancer

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Introduction: Breast cancer is an important public health problem worldwide. The HER2 /neu protooncogene is amplified and overexpressed in approximately 25-30% of invasive breast carcinomas. DNA topoisomerase 2-alpha enzyme controls and alters the topologic states of DNA during transcription. TWIST expression in breast tumors correlate with increased disease recurrence and poor disease-free survival. Steroid receptor genes family members such as the RARβ2 and ESR1 genes are methylated and silenced in a fraction of breast cancer.
Method: In this study we analysed retrospective HER2/neu, TOP2A gene and Chromosome17 copy number alterations by fluorescence in situ hybridization (FISH) in primary tumor core biopsies from 100 high-risk primary breast cancer patients (tumors ≥2 cm and/or lenfatic metastase and/or distant metastases and/or under 40 years) . The methylation levels of the TWIST, RARβ2 and ESR1 gene promoters were assessed Methylation Sensitive High Resolution Melting Analysis (MS-HRM).
Results: In our study, HER2/neu amplifications were identified in 25% and TOP2A amplifications in 24% and deletions in 6% of patients. HER2/neu and TOP2A amplifications are found to be associated with IDC tumor type and high grade also HER2/neu amplifications is associated with PR(-), TOP2A amplifications is associated with ER(+). TOP2A deletions is associated with ER(-) and PR(-). Polysomy17 was present in 23% and monosomy 12% of patients. TWIST, RARβ2 and ESR1 methylation frequencies were 24%, 90% and 69% respectively.
Conclusions:. Our study is important as being the first study that analyzes association between HER2/neu, TOP2A gene copy numbers and TWIST, RARβ2 and ESR1 gene promotor methylation status in Turkish population.

PM12.030

Exome analysis of families with hereditary breast and ovarian cancer (HBOC) to identify new candidate genes related with breast cancer development

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Introduction: Breast cancer (BC) is the most common type of cancer in women worldwide. Hereditary Breast and Ovarian Cancer (HBOC) accounts for ~10% of BC cases, a fraction which stems from the genetic effects of rare alleles. At least 20 genes have been linked to BC susceptibility and a high fraction of genetic effects remains to be explained. The aim of this project is to identify genetic variants linked to HBOC through exome sequencing in Greek patients that were found to be negative for mutations in known candidate genes.
Materials and Methods: Exome sequencing was performed on a total of 50 HBOC patients and informative relatives. Exome capture was through the Ion TargetSeq Exome Capture kit and sequencing was performed on the Ion Proton platform. An analytical pipeline was compiled adapting the GATK software package to IP data.
Results: We detected an average ~55,000 variants per exome. We filtered our data using criteria based on relatedness of individuals sequenced, BC segregation in families, minor allele frequency of variants and in silico functional evaluation. This resulted into collection of shortlisted variants amounting to ~100 per family.
Conclusion: Preliminary results have highlighted candidate variants in PRDM2, EME1, XRRC1 and GAB4. Genetic variants have been validated through Sanger sequencing. Further functional validation is necessary to confirm the pathogenicity of the candidate variants. This project is funded by SYNERGASIA 2011 (NSRF 2007-2013, code: SYN11_10_19, NBCA).

PS12.031

Estrogen withdrawal, breast cell transformation, and breast cancer risk in women with the KRAS-variant

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Background:
The polymorphism rs61764370 is a functional variant in the let7a-binding site of KRAS that is associated with increased cancer risk, particularly breast and ovarian. This risk may be modified by menopausal status and HRT use.
Aim:
To evaluate the effect of estrogen exposure and withdrawal on development of breast cancer in patients with the KRAS-variant (vKRAS).
Methods:
Isogenic mammary (MCF10A) cell lines with and without vKRAS were cultured and observed for oncogenic transformation in charcoal-stripped media following oestrogen withdrawal and restoration. Cells with and without vKRAS were examined for epithelial-mesenchymal transition(EMT) by western blot, immunoflourescence, and relative quantification of EMT-associated miRNAs. The effect of the vKRAS on disease phenotype was investigated in a cohort of patients with breast cancer, comparing variant and wild-type genotype carriers. A case-control analysis was also performed using a control group of unaffected vKRAS carriers. Data was collected with respect to pathological characteristics, reproductive risk factors and anthropomorphic measurements.
Results:
Isogenic cell lines with vKRAS showed evidence of EMT, with relative overexpression of vimentin and fibronectin. Acute estrogen withdrawal by addition of tamoxifen to charcoal-stripped media led to 7.9-fold increase in oncogenic transformation, with reduction in colony formation after restitution of oestrogen. Affected vKRAS carriers were significantly more likely to have oophorectomy pre-diagnosis than wild-type patients(p=0.033). Affected vKRAS carriers with breast cancer had lower BMI(p<0.01) than vKRAS unaffected controls. HRT-discontinuation in vKRAS carriers was significantly associated with post-menopausal triple negative breast cancer.
Conclusions: Estrogen withdrawal and a low estrogen state appear to increase breast cancer and predict aggressive tumor biology in women with variant KRAS.

PM12.032

Clonal expansion and linear genome evolution through breast cancer progression from pre-invasive stages to asynchronous metastasis

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Evolution of the breast cancer genome from pre-invasive stages to asynchronous metastasis is complex and mostly unexplored, but highly demanded as it may provide novel markers for and mechanistic insights in cancer progression. The increasing use of personalized therapy of breast cancer necessitates knowledge of the degree of genomic concordance between different steps of malignant progression as primary tumors often are used as surrogates of systemic disease. Based on exome sequencing we performed copy number profiling and point mutation detection on successive steps of breast cancer progression from one breast cancer patient, including two different regions of Ductal Carcinoma In Situ (DCIS), primary tumor and an asynchronous metastasis. We identify a remarkable landscape of somatic mutations, retained throughout breast cancer progression and with new mutational events emerging at each step. Our data, contrary to the proposed model of early dissemination of metastatic cells and parallel progression of primary tumors and metastases, provide evidence of linear progression of breast cancer with relatively late dissemination from the primary tumor. The genomic discordance between the different stages of tumor evolution in this patient emphasizes the importance of molecular profiling of metastatic tissue directing molecularly targeted therapy at recurrence.

PS12.033

Screening of HELQ in breast and ovarian cancer families

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Several risk-alleles have been identified for breast and ovarian cancer predisposition and most of them encode proteins that function in DNA repair. In the Finnish population, recurrent founder mutations have been observed in most of the susceptibility genes whereas in out-bred populations each gene may harbor rare unique mutations. A prospective candidate for breast and ovarian cancer susceptibility is the HELQ helicase that has a role in the resolution of DNA interstrand cross-links. HELQ interacts with the RAD51 paralog complex BCDX2. Two components of the complex, RAD51C and RAD51D, increase the risk of ovarian cancer, while the other two, RAD51B and XRCC2 have been associated with breast cancer risk. To investigate the role of *HELQ* in cancer predisposition and to identify putative recurrent founder mutations, we screened the *HELQ* gene for germline variation in 182 Finnish familial breast or ovarian cancer patients. To study the role of common variation in the gene, we performed haplotype analyses for 1517 breast and 308 ovarian cancer cases and 1234 population controls. No deleterious mutations were identified among the patients and the haplotype distribution did not differ between breast or ovarian cancer cases and population controls. Our results indicate that *HELQ* is not a major breast and ovarian cancer susceptibility gene in the Finnish population. However, we cannot rule out rare risk-variants in the Finnish or other populations.
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PM12.034

Clinical Characteristics of Breast Cancer in Women with a PALB2 Mutation

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PURPOSE: To estimate the lifetime risk of breast cancer in Polish women who carry a founder mutation in the PALB2 gene and to establish the clinical characteristics of breast cancers in patients with a PALB2 mutation.
PATIENTS AND METHODS: 12,529 breast cancer patients from Poland and 4,702 controls were genotyped for two deleterious mutations in PALB2 (c.509_510delGA and c.172_175delTTGT). Among breast cancer patients, the ten-year survival of carriers of a PALB2 mutation was calculated and compared with that of non-carriers.
RESULTS: A truncating PALB2 mutation was found in 116 breast cancer patients (0.93%) and in 10 controls (0.21%; OR = 4.39; 95% CI = 2.3 to 8.4; p < 0.0001). The ten-year survival of women with breast cancer and a PALB2 mutation was 49%, compared to 76% for women without a mutation (HR for death = 2.14; 95% CI = 1.55 to 2.95; p < 0.0001). Among the 2,065 patients who died, 38 women (1.8%) carried a PALB2 mutation.

CONCLUSION: Women with a PALB2 mutation face increased risks of breast cancer and of death from breast cancer. Given the high incidence and case-fatality associated with mutations in this gene, preventive mastectomy should be discussed with unaffected women who are found to carry a PALB2 mutation.
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PS12.035

Genetic predisposition to bilateral breast cancer: bioinformatics challenges in whole exome sequencing data analysis

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Contralateral breast cancer (CBC) is strongly associated with a heritable predisposition. The Women's Environmental Cancer and Radiation Epidemiology (WECARE) Study investigates susceptibility genes for CBC in women who received radiation treatment for the primary tumour. Whole exome sequencing (WES) was performed in DNA samples extracted from the blood of 256 women with unilateral BC and 256 closely matched women with CBC who did not have *BRCA1*, *BRCA2* and *PALB2* mutations based on previous screening. Matching was prioritised by radiation exposure, age at onset, latent period between diagnoses and family history of breast cancer. Libraries were prepared using Illumina Nextera Rapid Capture Exome kits. Sequencing was performed on Illumina HighSeq2500 machines to ~50x depth. Initial findings will be presented and challenges in data analysis discussed. The focus will be on quality control during data acquisition and current bioinformatics and statistical approaches that can be employed to detect genetic alterations associated with CBC. The quality control analysis found that longer reads may reduce efficiency of sequencing when used with Nextera library preparation kits because of the overlap in reads. Regarding the main analysis pipeline we will discuss (i) concordance assessment with previously obtained GWAS data (ii) performance and limitations of available tools for detecting structural variations and (iii) tools and resources required for adopting GRCh38 assembly in WES data analysis. Finally, we will compare statistical models that can be employed to detect single-genes as well as multi-gene patterns associated with unilateral BC versus CBC in the WECARE study.

PM12.036

Clinical utility of a breast cancer gene panel to improve clinical management of familial breast/ovarian cancer families

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Germline mutations in the BRCA1 and BRCA2 breast cancer susceptibility genes account for up to 20% of inherited breast cancer cases. This leaves a sizable fraction of familial cases where the heritability link remains unidentified meaning affected families may not receive appropriate clinical management including predictive testing for at-risk family members. In recent years a number of other breast cancer susceptibility genes have been identified. Although they may be individually less prevalent, together they promise to account for a significant proportion of familial cases. An extended breast cancer gene next generation sequencing strategy has therefore been developed to determine the role these genes play within the clinical diagnostic setting. A large cohort of families previously found not to have a pathogenic BRCA1/2 gene mutation were screened for mutations in an additional 10 genes (ATM, BRIP1, CDH1, CHEK2, PALB2, PTEN, RAD51C, RAD51C, STK11, and TP53) enriched using the Illumina TruSight Cancer Panel. Sequencing data was processed using an in-house bioinformatics pipeline optimised for sensitive indel detection. To date this new strategy has been used to screen 121 patients. Twelve patients (10%) were identified as having a class 4/5 pathogenic mutation in ATM, CHEK2, PALB2, RAD51D and TP53 according to criteria recommended by the ACGS Evaluation of Pathogenicity Best Practice Guidelines, including confirmation by RNA analysis. Given the limited penetrance of some of these genes segregation studies are offered to further support pathogenicity within individual families. This study is already indicating that this approach will improve the management of breast cancer families.

PS12.037

Tumor microRNA expression profiling identifies circulating microRNAs for earlier breast cancer detection

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Background: The identification of novel biomarkers for early detection of breast cancer would be a great advance. Due to their role in tumorigenesis and stability in body fluids, microRNAs (miRNAs) are emerging as a promising diagnostic tool. The aim of this study was to identify miRNAs deregulated in breast tumors and evaluate the potential of circulating miRNAs in breast cancer detection.
Methods: MiRNA expression profiling of 1919 human miRNAs was conducted in paraffined tissue from 122 breast tumors and 11 normal breast tissues. Differential expression analysis was performed generating a microarray classifier. The most relevant miRNAs were analyzed in plasma from 26 healthy individuals and 83 breast cancer patients (36 pretreated and 47 postreated), and validated in 116 healthy individuals and 114 pretreated patients.
Results: We identified a large number of miRNAs deregulated in breast cancer and generated a 25-miRNA microarray classifier that discriminates breast tumors with high sensitivity and specificity. Ten miRNAs were selected for further investigation in plasma and 4 of them (miR-505-5p, miR-125b-5p, miR-21-5p and miR-96-5p) were found to be significantly overexpressed in pretreated breast cancer patients when compared with healthy individuals in two different series of plasma. MiR-505-5p and miR-96-5p were the most valuable biomarkers (AUC=0.72). Moreover, the levels of miR-3656, miR-505-5p and miR-21-5p decreased in a group of treated patients.
Conclusions: MiRNAs can discriminate breast tumors in peripheral blood. The identification of deregulated miRNAs in plasma of breast cancer patients supports the use of circulating miRNAs as a novel method for early breast cancer detection.

PM12.038

Not HOXB13 p.G84E, but p.R217C appears to be associated with increased breast cancer risk in the Dutch population

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HOXB13 plays an important role in breast tumorigenesis as high expression of *HOXB13* predicts poor outcome and adverse response to endocrine therapy. At the genetic level, the *HOXB13* p.G84E mutation confers a four to five-fold increased prostate cancer risk and thus *HOXB13* p.G84E might also be associated with increased breast cancer risk. Previous studies investigating this association, however, reported conflicting results. Therefore, we now have comprehensively interrogated the entire *HOXB13* coding sequence for mutations in 1250 non-*BRCA1&2* familial breast cancer cases and 800 controls. In total, seven missense mutations were identified of which five were seen only once. The p.G84E mutation, however, was identified in 4 (0.33%) of 1215 cases and 6 (0.79%) of 759 controls. Another recurrent mutation, p.R217C, was found in 6 (0.50%) of 1206 cases and 1 (0.13%) of 765 controls. Because both mutations were predicted to be damaging, we further evaluated their association with breast cancer risk by expanding our case-control study to include a total of 4520 non-*BRCA1&2* familial breast cancer cases and 3127 controls. Custom genotyping revealed that p.G84E was present in 22 (0.50%) of 4415 cases and 22 (0.71%) of 3089 controls (OR=0.70, 95% CI=0.39-1.26, *P*=0.23). The *HOXB13* p.R217C mutation, however, was identified in 16 (0.36%) of 4444 cases and 3 (0.10%) of 3077 controls (OR=3.70, 95% CI=1.08-12.72, *P*=0.033, P_{adj}=0.066). Our results show that *HOXB13* p.R217C rather than p.G84E appears to be associated with breast cancer risk. *HOXB13* p.R217C is thus a putative novel moderate-risk breast cancer susceptibility allele.

PS12.039

Identification Of Rare And Novel Alleles In Ffpe Tumor Samples Using Laser Capture Microdissection (Lcm) And Ampliseq™ Sequencing Technologies

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Tumors are becoming recognized as genetically heterogeneous masses of cells with different clonal histories. Identifying the mutations present in these heterogeneous masses can lead to important insights into the future behavior of the tumor and possible intervention mechanisms. However, the rarity of pathogenic mutations in small subsets of cells can make identification of such alleles difficult. In this study, we demonstrate a complete workflow that facilitates the identification of rare and novel alleles from FFPE tumor sections. We collected small regions with different cellular morphologies from lung tumor samples using laser capture microdissection, extracted both DNA and RNA from these regions, and characterized mutations present and transcript abundances by using Ampliseq™ targeted sequencing. We show that LCM facilitates the detection of alleles that are not detectable in macrodissected tissue scrapes. We also show that different regions of a tumor have very different patterns of alleles detectable and have a great deal of genetic diversity. Finally, we show that RNA expression patterns are also clearly different in the different regions. Interestingly, dissected regions with similar gross tissue morphologies display differences in alleles present and RNA expression patterns. These results suggest the best way to analyze mutations present in a tumor is to microdissect different subregions of the tumor, and using Ampliseq™ panels to identify the alleles present in those subregions.

PM12.040

Case-control genetic association study of urothelial bladder carcinoma among Pakistani population

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A case-control genetic association study on Pakistani urothelial bladder carcinoma (UBC) patients (N = 200) and controls (N = 200) was conducted. VNTR polymorphism of eNOS; Alu repeat variation of ACE; null polymorphisms of GSTT1 and GSTM1 and selected common variants of GSTP1, MTHFR, PSCA, TNFα, p21, p53, CYP1B1, XPD, XRCC1, CAV1, PON1, IGFBP3, VEGFA, LEP, LEPR, PPARγ genes as well as 8q24.21 locus (rs9642880 and rs6983267) were analyzed for an overall risk assessment and with respect to smoking status, tumor grade and stage.
Variants of GSTM1, LEPR, ACE, PSCA, rs9642880 and rs6983267 were found to be associated with higher risk while IGFBP3 variant and haplotypes of CAV1 and MTHFR with reduced risk of UBC in the overall comparison.
CYP1B1, p21, ACE and rs9642880 conferred a high risk to smokers while LEPR and PSCA variants to non-smokers. In contrast, IGFBP3 variant and CAV1 haplotypes conferred protection to non-smokers.
GSTM1, LEPR and rs9642880 were found to be associated with enhanced risk of low grade and non-invasive UBC; while GSTT1, CAV1, PSCA and PPARγ with an elevated risk of high grade and invasive UBC. IGFBP3 variant protected against low and high grade tumors and non-invasive disease. Different haplotypes of MTHFR were found to confer a high risk of non-invasive tumor while providing protection against invasive.
In brief, genetic associations to UBC susceptibility, few being novel, were observed in the present study, which to the best of author's knowledge, is the first attempt to reveal genetic epidemiology of UBC exclusively among Pakistani population.
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PS12.041

Case studies demonstrating that use of a large, augmented panel to sequence cancer genes to high depth enables clinically-relevant variant identification

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Introduction: Genetic lesions in cancer can determine prognosis and have diagnostic value. Next-generation sequencing facilitates the detection of a wide array of somatic mutations, copy number alterations (CNAs), and gene fusions in a single assay. With increased sequencing depth, mutations present in only a fraction of the neoplastic tissue can be identified. Detection of these mutations after sequencing DNA and RNA involves a complex set of variant detection algorithms tailored specifically for cancer analysis.
Materials and Methods: We designed a cancer panel using an augmented target enrichment strategy optimized for even coverage across the entire span of gene content to detect small variants, CNAs, and gene fusions. The panel includes over 1,500 cancer genes and micro-RNAs. Very high depth (>500x) sequencing detects low representation alleles. We tested the panel and pipeline using over 30 different cancer samples including cancer cell lines, engineered cells containing specific cancer mutations, and primary tumors in FFPE.
Results: We demonstrate that this cancer gene panel and analysis strategy is able to detect mutations of each major type with high sensitivity and positive predictive value. We saw greater than 99% sensitivity for small variants down to 5% allele frequency. We also detected known gene fusion events including *EML4-ALK* and *BCR-ABL1* accurately in all 20 cancer samples with known fusions. Moreover, the assay was able to identify CNAs consistent with the known state, including detection of *EGFR* amplification, *EGFR*vIII, *PTEN* loss, and *p16* loss in known cases.
Conclusions: The high depth, augmented cancer gene panel assay is able to comprehensively identify cancer mutations with high accuracy.

PM12.042

Exome sequencing and molecular heterogeneity in a cohort of Anaplastic Large Cell Lymphoma

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Exome sequencing has been conducted on a panel of frozen paediatric Anaplastic Large Cell Lymphoma (ALCL) tissue samples. Exome sequencing was conducted using an Illumina MiSeq. Variants (indels/SNPs) were identified using the GATK (Genome Analysis Tool Kit) pipeline using the hg19 reference genome. A molecular characterisation has been undertaken to assess inter-patient molecular tumour homogeneity/heterogeneity and data is presented on these initial findings. One patient had tissue samples derived from a primary, and two relapse cases. SNPs and indels were detected that were concordant between all three samplings. Analysis of temporal changes between the primary tumour and second recurrence also identified SNPs and indels that were restricted only to the secondary recurrence. In-silico tools were utilised to examine potential effects upon protein function including frameshift mutations caused by indel. Several genes were identified including RBM33, SEMA5B and ZNF155 that had high impact changes to their genetic sequence with potential downstream consequences to protein structure and function. These studies provide the framework for a detailed molecular analyses of exonic dynamics and molecular evolution of ALCL tumours.

PS12.043

Personalized cancer mutation panel by next generation sequencing for cancer prediction

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Objective:
Next-generation sequencing (NGS) allows for high-throughput sequencing analysis of numerous regions of the human genome. We explored the use of targeted mutation detection by NGS for simultaneous genetic testing for multiple caner genes.
Methods: We used a personalized cancer mutation panel to target more than 2,800 mutations in the 50 key cancer genes. NGS was performed to jointly sequence captured DNA individually for 2 healthy cases.
Results: Using targeted mutations sequencing, we achieved an average sequence depth of ~1000× per base. We analyzed DNA from 2 unrelated individuals and identified a heterozygous c.1151T>A (p.V384D) mutation of the MLH1 gene in case 1, which is highly associated with pancreatic cancer; a heterozygous mutation c.2472C>T (p.V824V) of the PDGFRA gene was detected in case 2, which is highly associated with endometrium cancer.
Conclusion: The personalized cancer targeted mutation detection NGS panel allows simultaneous testing for multiple genes with high accuracy. Using this approach can fast identification of mutations in key cancer genes and can provide important individual information of tumor development and cancer prediction.

PM12.044

From national guideline recommendations to familial cancer risk assessment decision support in primary care: UK experience

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Introduction: The English national guidelines on managing women with a family history of breast cancer provides criteria for specialist care referral and for patients that can be followed-up in primary care. Also there is a category, with unclear family histories, where the guidelines recommend that patients are not referred directly but discussed with specialists. We have explored the nature of family histories that fall into this category and how to integrate this information into primary care decision support software. The guidelines also recommend genetic testing, hence referral, when probability of BRCA1/BRCA2 is greater than 10%.
Materials and Methods: In our exploratory trial all women aged 30 to 60 in four General Practices, in Central England, were invited to complete a family history questionnaire. The family histories were assessed against national guidelines and Manchester Scoring System.
Results: Currently, 13.1% (126) of 963 women completing the questionnaire were recommended by guidelines to discuss with familial cancer specialist. Of these 126 participants, 9.5% (12) of pedigrees had greater than 10% probability of BRCA1/BRAC2, based on Manchester Scoring System. Further, the specialist identified that the guideline's referral criteria had not fully taken account of familial risk of related cancers and age of onset in relatives.
Conclusion: To operationalise national guidelines and implement decision support software in primary care, uncertain family histories need to be reduced. This could be achieved by combining national guidelines with consensus opinion of specialists and key attributes of evidence-based referral tools, such as Manchester Scoring Systems.
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PS12.045

Intratumoral Genetic Heterogeneity in Clear Cell Renal Cell Carcinoma

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Clear Cell Renal Cell Carcinoma (ccRCC) is well known for the heterogeneity of its clinical, histologic, and genetic profiles. We optimized the preparation of DNA samples from paraffin material to identify the genetic profile of different tumour areas of one case of ccRCC. DNA was isolated from paraffin sections of four tumour areas and normal kidney cortex. The morphological variety among different areas of the tumour has been documented. We performed pre-analytical RT-PCR to measure the quality of amplifiable DNA input. Aliquotes of 100 ng of DNA were subjected to whole exome sequencing (Agilent SureSelect All exon V2®, Illumina HISEQ 2500®) and targeted exome sequencing (NugenOvation® Target Enrichment System) as an alternative approach to obtain reliable sequence data from FFPE material. Based on published data a minimum Quantitative Functional Index (QFI) of 6-7% from RT-PCR is needed to achieve 90% confirmed variants on sequencing data. The QFI of our FFPE DNA samples showed values of 8.9-12.6%, indicating that the quality of our DNA samples was acceptable for further analysis. The quality control report of sequencing data showed a 20x coverage of 68-86%. Preliminary analyses of the sequence data identified 151 somatic mutations, including 3 indels. In these preliminary data we see a unique genetic profile shared by areas which have similar tumour histological grade. The observed mutational heterogeneity present among different areas of ccRCC may be important in cancer development.

PM12.046

Identification of pathogenic CDH1 mutations in three families in DNA extracted from FFPE tissue from deceased family members with suspected hereditary diffuse gastric cancer

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Introduction: Hereditary diffuse gastric cancer (HDGC) is a cancer susceptibility syndrome caused by pathogenic mutations in *CDH1*. HDGC is inherited in an autosomal dominant manner and mainly characterized by a high cumulative risk of developing diffuse gastric cancer (approximately 80% for both sexes) and lobular breast cancer (approximately 50% for women). As with other hereditary cancer syndromes, the task of identifying the disease-causing mutation in a family has often been difficult without affected living family members. Identifying a new family mutation in tissue-derived DNA from deceased is technically challenging for various reasons, and because of that, this diagnostic approach has not been offered on a regular basis by diagnostic departments or companies.
Materials and Methods: DNA was extracted from 9x15 µm FFPE tissue sections per sample, and DNA integrity was estimated. DNA samples were subjected to HaloPlex Target Enrichment using a custom design including *CDH1* (Agilent Technologies). After enrichment, HaloPlex libraries were diluted, pooled, denatured and 10 pM library pool was subjected to paired-end (2x150 bp), single index (8 bp) DNA sequencing on a MiSeq (Illumina).
Results: We identified a pathogenic *CDH1* mutation in three different families with one or more deceased family members with suspected HDGC. In all three cases, the mutation was identified in DNA extracted from FFPE tissue from a deceased.
Conclusions: The possibility of identifying pathogenic mutations in deceased family members is a new and important tool in the process of determining or evaluating the risk of certain cancers of family members who receive genetic counseling.

PS12.047

A multiplexed amplicon sequencing technology for FFPE and circulating cell-free DNA

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Detection of somatic mutations is challenging since percent tumor content in clinical samples is variable and is compounded by tumor heterogeneity. Additionally, circulating cfDNA and FFPE samples are limited in quantity, and FFPE samples can be damaged. To address these challenges, we developed a single tube, multiplexed amplicon sequencing method that employs hundreds of primer pairs for amplification of target loci, producing ready-to-run libraries for Illumina sequencing. The two-step method- multiplexed PCR followed by a 10 minute adapter ligation- results in amplicons that are 120-160 bp in length, enabling amplification and variant calling from cfDNA-sized DNA fragments or damaged FFPE DNA. An oncology panel was developed to target known, clinically relevant mutations in 56 genes. The panel design encompasses single exons (e.g. BRAF) and comprehensive coding exon coverage of entire genes (e.g. TP53), depending on the allele distribution across each gene. To validate this panel, a cohort of control and clinical samples with pre-validated genotypes was tested using 10ng of input DNA. Variant calling was performed using GATK and LoFreq. Robust detection of 5% mutant frequency was observed, and the limit of detection was as low as 1% mutant frequency. The percent on-target bases and coverage uniformity were both >95%, where uniformity is defined as the percent bases covered at >20% of the mean coverage. These results indicate that this multiplexed amplicon panel is an excellent tool to assess multiple oncogenes in limiting clinical samples, enabling high throughput, cost effective NGS analysis.

PM12.048

A circulating cell free DNA targeted sequencing workflow - from isolation through validation

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Circulating cell free DNA has been shown to have the potential as a non-invasive substrate for the detection of cancer and its progression as well as the determination of therapeutic resistance. As circulating tumor DNA is often present at low frequencies within circulating cell free DNA, targeted sequencing is an optimal tool for mutation detection. Here, we demonstrate a complete workflow from isolation through validation of circulating tumor DNA.
We have optimized an easily automatable protocol using magnetic beads to isolate circulating cell free DNA. It is also scalable for any input volume and can elute in volumes down to 15 µL resulting in no loss of low frequency alleles. We demonstrate comparable performance between this bead based isolation and column based isolation.
We then completed molecular characterization of the isolated circulating cell free DNA using the multiplexing capabilities of AmpliSeq™ and the Ion Torrent™ platform for targeted sequencing of 50 genes of interest. We demonstrate good reproducibility of amplicon representation as well as allelic frequencies. We have determined the limit of detection of hotspots circulating cell free DNA on the Ion Torrent™ platform to be below 1%. We further demonstrate proof of principle of this workflow on circulating cell free DNA and matched FFPE samples complete with TaqMan® validation.
Our results validate the accuracy and ease of our workflow. This protocol, from isolation through targeted sequencing and validation, will not only re-

sult in a simple sample preparation for circulating cell free DNA but also facilitate rapid mutation detection to advance cancer research.

PS12.049

Comprehensive genomic characterization of intrahepatic cholangiocarcinoma

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Intrahepatic cholangiocarcinoma (ICC) is a cancer arising from the epithelium of the biliary tracts located within the liver. ICC represents the second most common primary liver malignancy with significant rise in incidence over the past three decades. The molecular mechanisms underlying ICC tumorigenesis are not well understood, although recently the first whole-exome sequencing (WES) studies have been performed on tumor samples derived from patients with Asian descent.
In order to identify common genomic alterations driving ICC development, we conducted the largest WES study on patients of Central European origin. We collected tumor and matching control tissue samples from 38 ICC-patients and performed WES as well as microarray genotype and gene expression analyses. The data were used to obtain genomic profiles comprising somatic mutations, copy number aberrations and gene expression patterns.
In line with previous studies, somatic mutations affecting tumor suppressor genes implicated in chromatin remodeling including ARID1A (14%), BAP1 (14%) and PBRM1 (11%) could be identified. In addition, the oncogenes IDH1 and IDH2 (14%) showed frequent mutations and may also play an important role in the development of ICC. Other frequent alterations affected genes implicated in the p53 and MAPK signaling pathways. Integrative analysis of the data sets further revealed ICC driver gene candidates. Their role in the development of ICC will be examined by functional studies and by screening larger tumor collectives in the near future.
In conclusion, the results of the current study confirm and expand the genomic landscape of ICC. This, finally, will help to find new therapeutic approaches for the treatment of this orphan cancer.

PM12.050

Molecular characterization of commonly used chondrosarcoma cell lines

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Chondrosarcomas (CHS) are malignant tumours of bone that produce hyaline cartilage matrix. Primary CHS is the second most frequently primary malignant tumour of bone after osteosarcoma, and represents about 25% of bone sarcomas. Prognosis is strongly correlated with histological grading. Grade I CHS typically does not metastasize, in contrast with grade II and III CHS. The ten-year survival is 83% for patients with grade I, 64% for grade II and 29% for grade III. CHS is highly resistant to both chemotherapy and radiation, making surgical resection the only curative treatment.
Due to the limited amount of primary tumour specimens, inclusion of in vitro models for CHS investigation is of high importance. In addition, as comprehensive genomic analyses of CHS become available, there is a growing need for corresponding cell models to performed functional analysis of new identified variants. To date, commonly used CHS cell lines has been only partially screened by target-genes approaches.
Our study is the first which aims to extensively characterize commonly used human CHS cell lines by exome sequencing to identify somatic variants and biological pathways potentially involved in the tumorigenesis. We generate an average of 4.9 Gb of sequence per cell line with a mean depth of 67-fold and with 90% of the targeted bases which have a QPhred score > 30. On average, 56 000 variants were identified per exome, including 1700 new variants.
In conclusion, this study will be useful to highlight genetic alterations associated with malignant transformation and potential molecular therapeutic targets.

PS12.051

Loss Of The Y Chromosome In Myelodysplastic Syndromes

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Introduction: The clinical association between loss of the Y chromosome and MDS has been debated, because both phenomena are related to aging. The objective of this study was to asses if the the mean age is related to the loss of the Y chromosome.
Material and Methods. From 1986 to July of 2014, 875 patients were diagnosed of Myelodysplastic Syndrome (MDS) in the Hospital Clinic of Barcelona and 275 (55%) were male. ANOVA was used for age comparison between groups.
Results. Twenty-six (6%) patients showed a loss of the Y chromosome, in most of them as a sole abnormality. According to the WHO classification about half of these 28 patients were affected of refractory cytopenia with multilineage dysplasia (RCMD) (n=16). The mean age in patients with loss of the Y chromosome was 77 years old (95%CI: 74 to 80), significantly higher than that observed in patients with either a normal karyotype (71 y.o.; 95%CI: 70 to 73) or with an abnormal karyotype without loss of the Y chromosome (61 y.o.; 95%CI: 65 to 70).
Conclusion. Based on our results, the loss of the Y chromosome is observed more frequently in a group of elder MDS patients.

PM12.052

Potential new player in prostate cancer susceptibility and survival in Finland

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CIP2A (Cancerous Inhibitor of PP2A) gene has been demonstrated as potential cancer susceptibility gene. Aim of this study is to explore the population diversity of CIP2A rs2278911 common variant in Finnish, to elucidate its role in prostate cancer (PCa) susceptibility, survival, potential in development of aggressive disease, association with specific, clinically different subtypes of PCa.
We genotyped 2738 men with PCa, 2427 healthy controls within the Finnish Genetic Predisposition to Prostate Cancer Study (iCOGS) using Illumina iSelect custom SNP genotyping platform.
This is the first comprehensive case-control study assessing the prevalence of the CIP2A R229Q variant, which revealed that the ancestral C allele is the major allele, the variant T is the minor allele in Finnish population. The overall minor allele frequency in entire sample set 13.8%. Notably, CIP2A rs2278911 minor allele showed slightly, but not significantly protective effect in PCa cases (13.2%) relative to controls (14.3%) (OR, 0.912; 95% CI, 0.815-1.020; p=0.106). This is underlined by the fact that the minor allele significantly confers to belong to the least serious combined PCa stage group (OR, 1.212; 95% CI, 1.010-1.454; p=0.039). Association between CIP2A rs2278911 variant and the development of castration resistant PCa, clinically detected, progressed cases, PCa cancer specific death was not revealed in Finnish samples. Survival between PCa diagnosis and progression, progression and overall or PCa-specific death were not affected by CIP2A R229Q variant.
In genetically homogenous Finnish CIP2A rs2278911 minor allele alone suggests slight protection against PCa, which need to be ascertained in pooled European samples.

[*]Members from the Prostate Cancer Association Group to Investigate Cancer Associated Alterations in the Genome (PRACTICAL) consortium are provided in the Supplement/foot notes. Information of the consortium can be found at http://practical.ccge.medschl.cam.ac.uk/.

PS12.053

Sensitive Mutation Detection By Sequencing Circulating Cell-Free DNA

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Circulating DNA, the cell-free DNA (cfDNA) in serum or plasma, has become a powerful tool used for Non-Invasive Prenatal Testing (NIPT) as well as cancer liquid biopsy. It has been shown that the quantity, integrity, as well as the mutation contents of the cfDNA in cancer patients could differ from that in healthy controls and therefore serve as biomarkers for cancer diagnosis, prognosis, and stratification. High-throughput sequence analysis of the cfDNA with the rapidly developing next generation sequencing (NGS) technologies provides a highly sensitive method in detecting and characterizing somatic mutations in the cancer patients. However, the concentration

of the cfDNA in the serum is normally very low, which posts challenges in sequencing library construction. Here we describe an optimized workflow that combines high-efficiency NGS library construction, unbiased library amplification, and target enrichment to sensitively and reliably detect mutations in cfDNA samples.

PM12.054

Detecting hypomethylation in circulating, cell-free DNA to monitor cancer burden

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Circulating, cell-free DNA (cfDNA) is a powerful, non-invasive sample source that contains tumor associated DNA. Characterizing the genome-wide methylation state of cfDNA is a powerful and economical biomarker that can be used for providing an effective means to monitor disease and treatment efficacy. The challenges in performing deep sequencing of cfDNA include: a requirement for fast turnaround times due to sample degradation issues, limited sample material, short DNA fragments <170 bp, and limit of detection issues caused by both normal and tumor DNA being present. This study describes a novel library preparation suitable for the Illumina platforms that requires only 10 million sequencing reads making it cost-effective, sensitive, and specific to assess the methylation status of cfDNA.
To characterize the methylation status of cfDNA, NGS libraries were generated utilizing a chemistry that sequentially ligates the adapters to each end of the DNA molecules. As the library is generated after bisulfite treatment, a high recovery of DNA library molecules is observed, thus enabling high complexity library preparation from 5 ng of cfDNA.
Preliminary analysis of the hypomethylation status of the cfDNA from 8 cancer subjects ranged from 0.4% to 44% when compared to a set of healthy controls. The cfDNA sample with 0.4% hypomethylation originated from the plasma of a subject with a high-grade serous adenocarconima in the fallopian tube, and it believed that this tumor type sheds very little DNA into the blood compartment. The most hypomethylated cfDNA came from the plasma of subject with metastatic adenocarcinoma of the colon which had metastasized to the liver.

PS12.055

Circulating miRNA expression profiling in chronic lymphocytic leukemia (CLL) patients - preliminary results

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MicroRNAs regulate gene expression on post-transcriptional level. miRNA expression is altered in cancer, and its profiling helps to diagnose the disease, to stratify patients into risk groups, and to predict the response to treatment. Circulating miRNA molecules were identified in different body fluids, and in many cancer types their expression reflected expression in tumor cells.
In our study we used qT-PCR to assay peripheral blood serum of 22 CLL patients for the expression of 84 miRNAs which were associated with differentiation and maturation of B and T lymphocytes.
We have found that the general expression of examined miRNAs in CLL patients was lower when compared to the expression levels in serum of healthy volunteers. Only miR-32a-5p, miR31-5p, miR-155-5p, miR-150-5p, miR-15a-3p and miR-29a-3p were expressed on higher level. Considering prognostic factors, the most important differences in miRNA expression were observed among patients depending on B2M level, CD38 expression status, and aberrations of chromosomes 12 and 13. When the functional miRNA groups were considered, the important factors for all examined groups included age, CD38 expression, NOTCH1 status and the status of chromosomes 11 and 12. Interestingly, neither ZAP70 expression, nor TP53 gene status were differentiating factors.
Alterations in circulating miRNA expression in CLL patients affected miRNAs associated both with B and T lymphocyte differentiation, which proves the role of the latter cells in leukemogenesis. Further study utilizing larger test group of patients may warrant the identification of a panel of several circulating mRNAs which would be used at diagnosis for prognostic and predictive purposes.

PM12.056

A polymorphic GGC repeat in the NPAS2 gene and its association with melanoma

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Circadian rhythms are influenced by the expression of clock genes, which are controlled by a feedback mechanism that allows a rhythmic variation during 24 hours. Various evidences indicate that clock genes are important in neoplastic transformation. *NPAS2 (MOP4)* is a clock gene that can act as a tumor suppressor. We are searching for the presence of polymorphisms in the 5' region of *NPAS2*. By using Sanger sequencing and capillary electrophoresis, we found a polymorphic GGC repeat in the untranslated (first) exon of *NPAS2*. Allele and genotype frequencies of the GGC repeat were measured in 72 subjects affected by melanoma and 77 controls. In both groups four alleles were present, with 7, 9, 12 and 13 GGC repeats. Alleles 7 and 9 were the most frequent (either having a frequency > 40% in controls or melanoma subjects). In both groups allele and genotype frequencies were in Hardy-Weinberg equilibrium. In terms of allelic frequencies no statistical difference was found between melanoma and control subjects. In contrast, significant differences were found in genotype frequencies. In particular, the genotype 7/9 was more frequent in controls (57.1%) than in melanoma subjects (34.7%) (p: 0.0084); the genotype 9/9 was more frequent in melanoma subjects (26.3%) than in controls (9.0%) (p: 0.0087). No statistical difference was found for other genotypes. Therefore, the homozygous genotype of the 9 GGC repeat of the *NPAS2* gene could be a susceptibility factor for melanoma.

PS12.057

CANCER RISKS IN FAMILY MEMBERS OF CMMR-D PATIENTS

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Introduction: Biallelic germline mutations in the mismatch repair (MMR) genes cause a recessive form of childhood cancer that has been referred to as Constitutional Mismatch Repair Deficiency (CMMR-D) syndrome. Family members of CMMR-D patients are at risk of being a heterozygous MMR mutation carrier and thus for having Lynch syndrome (LS). The cancer risks for these family members have not yet been analyzed. It is expected that their cancer risk will be different than cancer risks reported before for LS families that were ascertained because of cancer in the family. CMMR-D families have not been ascertained because of cancer in the family, but because the index patient has a distinct phenotype.
Methodology: Data collection of CMMR-D families started in 2014 and is still in process. In the first half of 2016 a competing risks analysis will be performed to calculate cancer risks for family members up to the 6th degree. For family members of whom the carrier status is unknown, the probability of carriership will be computed based on the distance to obligate carriers and phenotypes in the family.
Results: Thus far we have collected data on 46 PMS2, 15 MSH6, 4 MSH2 and 6 MLH1 families of CMMR-D-patients including 1764 family members. This numbers includes 340 proven and obligate heterozygote mutation carriers, 104 proven non-carriers and 1220 non tested family members. Data analysis will be done at a preliminary basis.
Conclusion: Analysis of cancer risks in CMMR-D families are relevant for appropriate screening advice for family members and MMR mutation carriers detected through population based screening.

PM12.058

Systematic meta-analyses and field synopsis of genetic association studies in colorectal adenomas

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Background: Colorectal cancer (CRC) constitutes a major global public health challenge. Most CRCs develop from preneoplastic asymptomatic lesions known as colorectal adenoma (CRA). We have previously summarized the associations between common genetic variants and CRC in a field synopsis of genetic association and GWAS, but the genetic basis of CRA is less well documented. We now present the first synthesis of all published genetic association data for CRAs and the results of meta-analyses to summarise risk estimates.
Methods: Using Medline and the HuGENet phenopedia™, we identified and synthesized all published genetic association data for CRAs. We conducted meta-analyses of the identified studies and data from two GWAS to summarise risk estimates. We applied the Venice criteria and Bayesian False Discovery Probability (BFDP) to assess the levels of the credibility of associations.
Results: 9750 titles and abstracts were initially screened, and 1750 publications were identified for full text screening of which 130 articles met the inclusion criteria. Data were extracted for 181 SNPs in 74 genes. The variant at 8q24.21 (rs6983267) was considered as "highly credible" and *MTHFR* (C677T), *NAT1*, *NQO1* (Pro187Ser), and *TP53* (Arg72Pro) as "less credible".
Conclusion: The identification of genetic variants with influence on CRA risk may provide new insights into the fundamental biological mechanisms involved in early CRC development and help to inform future research. Further, CRA risk-associated SNP variants may also show utility in contributing to future risk scores for accurate population risk stratification which could be of potential value in improving CRC screening modalities.

PS12.059

Overexpression of HDAC3 in colorectal cancer

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Introduction: Histone deacetylase 3 (HDAC3) is involved in many important biological processes including transcriptional regulation, cell cycle progression and developmental events. Aberrant expression of HDAC3 is observed in several human cancers, indicating a critical role in carcinogenesis and a potential as a tumor marker in cancer. The aim of this study was to investigate the expression level of HDAC3 in colorectal cancer and its relationship with the clinicopathological features of patients.
Materials and Methods: Total RNA was first isolated from 48 pairs of colorectal cancer tissues and adjacent non-tumor tissues. Afterwards cDNAs were synthesized and the expression level of HDAC3 was quantified by real time PCR. The Correlation between the expression level of HDAC3 and clinicopathological features was studied and the capability of HDAC3 to function as a CRC tumor marker was also explored.
Results: Over expression of HDAC3 was observed in colorectal cancer tissue samples compared with their paired adjacent nontumour tissue samples (P=0.03). The Receiver operating characteristic (ROC) curve analysis on HDAC3 showed that the area under the ROC curve was high (0.72). There was no significant correlation between HDAC3 expression and clinicopathological features of patients.
Conclusions: These findings suggest that HDAC3 can serve as a novel prognostic indicator in colorectal cancer and may be a potential target for diagnosis and gene therapy.

PM12.060

Detection of copy number alterations in pediatric acute lymphoblastic leukemia using the 450k DNA methylation array

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Pediatric Acute Lymphoblastic Leukemia (ALL) represents about one quarter of pediatric cancers. Our previous study (Nordlund *et al.* Genome Biology, 2013) investigated differences in genome-wide DNA methylation patterns across the recurrent ALL subtypes. Here we focus on structural alterations and Copy Number Alterations (CNAs), which are important genetic factors in ALL. This is the first study that uses bioinformatic tools for CNA analysis in cytogenetic subtypes of pediatric ALL based on DNA methylation data.
The Illumina Infinium HumanMethylation450 Bead Array was applied for 756 diagnostic ALL samples and 50 controls. The signals from the array were first normalized using functional normalization and then segmented using the circular binary algorithm. Methylation data was analyzed using the R packages Minfi and CopyNumber450k. CNVs were detected using an in-house modified version of the CopyNumber450k package adapted to cancer samples, in particular within the high hyperdiploid subtype (>50 chromosomes).
The unbalanced cytogenetic aberrations characteristic of the recurrent ALL subtypes were observed. In many cases, our results showed higher sensitivity than traditional karyotyping, in particular in the detection of duplicated and deleted chromosomes. Data from CNAs will be compared across all patients to identify potential novel aberrations associated with clinical outcome of ALL. Paired samples from the same individual will be compared at different disease stages: diagnosis, relapse, and remission. Significant differences will be selected for further functional investigations.
The results suggest that the DNA methylation arrays can be used to detect somatic structural alterations and CNAs with high accuracy in ALL and other leukemias.

PS12.061

Cost-effectiveness of UGT1A1 genotyping before colorectal cancer treatment with irinotecan: A decision analytic model from the perspective of the German statutory health insurance

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Irinotecan is an anti-cancer agent that is used for the treatment of metastatic colorectal cancer. Although it prolongs survival, it can cause severe toxicity, especially diarrhea and neutropenia, in patients who carry the UGT1A1*28 allele. This study evaluates the cost-effectiveness of UGT1A1 genotyping prior to irinotecan-based chemotherapy in order to prevent adverse side effects from the perspective of the German statutory health insurance.
A decision-analytic Markov model with a life time horizon was developed. No testing was compared to (1) dose reduction of irinotecan-based chemotherapy and (2) administration of a prophylactic G-CSF growth factor for patients with a UGT1A1*28 variant. Probability, utility and cost parameters used in this study were extracted from published literature. Uncertainty was assessed by deterministic and probabilistic sensitivity analyses.
Strategy (1) dominated all remaining strategies. Compared to no testing, it resulted in only marginal QALY increases but a cost reduction of €600 per patient. Strategy (2) resulted in the same health gains but increased costs by €11,000. Deterministic sensitivity analysis shows that uncertainty for this strategy originated primarily from costs for irinotecan-based chemotherapy, from the prevalence of neutropenia among heterozygous patients, and from whether dose reduction is applied to both homozygotes and heterozygotes or only to the former.
This model-based synthesis of the most recent evidence suggests that pharmacogenetic UGT1A1 testing prior to irinotecan-based chemotherapy dominates non-personalized colon cancer care in Germany. However, as structural uncertainty remains high, these results require validation in clinical practice, e.g. based on a managed-entry agreement.

PM12.062

Diffuse large B cell lymphoma developing simultaneously in recipient and donor twenty two months after allogeneic bone marrow transplantation.

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Donor cell hematological neoplasms are rare complications arising after allogeneic hematopoietic stem cell transplantation (alloHSCT). We present an eight-year-old boy diagnosed with AML, who underwent alloHSCT a year later with his 42-year-old mother as HLA-matched donor. After twenty-two months, he presented with thigh and buttock pain; bone marrow (BM) showed 20%-30% blast-like cells, suggesting relapsed AML. However, BM cytogenetic analysis showed all cells to be of female (donor) origin. Morphology and immunostains on BM, skin and spinal lesions confirmed infiltration by diffuse large B-cell lymphoma (DLBCL). FISH on the skin biopsy showed all lymphoma cells with two copies of the X chromosome centromere and no Y centromere, confirming that the DLBCL cells were donor-derived. FISH on the spinal mass detected concurrent MYC and BCL6 rearrangements, consistent with "double hit" lymphoma. A month after the boy's lymphoma diagnosis, the donor was found with a large abdominal mass diagnosed as DLB-

CL ABC type, with evidence of a BCL6 but not a MYC rearrangement. The absence of a MYC rearrangement in the donor's tumour suggests that this was secondary to the BCL6 rearrangement, possibly arising after transfer into the recipient as a consequence of clonal evolution. It is possible that both the conditioning regimen pre-transplant and the replicative stress on the donor stem cells post-transplant had a role in the acquisition of this second event. This hypothesis is consistent with the fact that the donor achieved complete remission after six cycles of R-CHOP, while double-hit lymphoma is usually resistant to such aggressive treatments.

PS12.063

EGFR genomic variations in five different types of cancer - tissue microarray survey and potential for targeted therapy

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EGFR was the first receptor that has been directly connected to carcinogenesis. EGFR-targeted agents have been showed to have different response efficacy in anti-cancer therapy suggesting the role of specific indicators such as EGFR alterations in selection of appropriate targets for treatment.
We have successfully analyzed by FISH the copy number of EGFR in samples from five different tumor types, arranged in tissue microarrays: 1075 larynx, 355 ovarian, 239 colon, 144 lung and 86 uroepithelial cancers.
The copy number increases were detected to the highest proportion in lung cancer (22.2% of them), following by 10.7% of ovarian and 10.6% of larynx cancers, 4.7% of urothelial and in very low number of colon cancer (0.8%). In the last we have observed only EGFR gain whereas in uroepithelial and larynx cancer we have found mostly EGFR amplification.
Studying the correlation with tumor phenotype, we established that EGFR aberrations affect to the highest extent the group of squamous cell lung cancer (in 23.5% of cases), mixed and non-epithelial ovarian cancer (both in 13.3% of the cases), poorly differentiated and high stage uroepithelial tumors (14.3% and 12.9%, respectively). The study provided with data which could be useful in considering of anti-EGFR anti-tumor therapeutic strategy.

PS12.065

Exome sequencing and SNP arrays to confirm genetic instability increasing from premalignant to tumour cells in monoclonal gammopathies

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Whole exome sequencing (WES) and SNP arrays have opened a new landscape to study compreshensive tumour genetic architecture and its evolution during tumour associated processes at the genome-wide level. Transformation from monoclonal gammopathy of undetermined significance (MGUS) to multiple myeloma (MM) can be used as a unique model for cancer development studies as an analysis of pure tumour population in clearly clinically distinguishable states.
Overall, 33 and 69 MGUS patients were included in a WES and SNP array study, respectively. For WES, NEBNext kit and SureSelect Human All Exon V5 (Agilent) were used, samples were sequenced by HiSeq2000 (Illumina). Copy number alterations (CNAs) were tested by SurePrint G3 CGH+SNP, 4x180K (Agilent). Results were compared to 463 and 91 MM patients analysed by WES and SNP arrays, respectively.
CNAs and somatic gene mutations (SNVs) were detected in 68% (47/69) and 100% (33/33) of MGUS patients in comparison to 100% (91/91, $p<10^{-4}$) and 100% (463/463) of MM patients, respectively. However, overall number of both CNAs and SNVs per patient was significantly lower in MGUS (CNAs: median 2, range 0-15; SNVs: median 89, range 9-315) than in MM (CNAs: median 16, range 2-49, $p<10^{-18}$; SNVs median 123, range 1-897, $p<10^{-4}$). We proved that complex genetic instability is formed before tumour clinical manifestation at the gene level followed by the chromosome level. Then, the number of random genetic hits increases to form a landscape for significant oncogenic hits driving the premalignancy transition to a clinically manifested tumour disease.
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PM12.066

Gene panel analysis in Spanish breast and ovarian cancer families testing negative for BRCA1 and BRCA2

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Introduction: Hereditary breast and ovarian cancer (HBOC) is a heterogeneous genetic condition. Susceptibility cancer gene panels allow simultaneous analysis of multiple genes and broaden genetic diagnosis compared to sequential single testing.
Patients and Methods: Two-hundred BRCA1/2-negative patients from HBOC families were enrolled for massive sequencing of 98 cancer susceptibility genes. Inclusion criteria were BC <35y, presence of OC <50y or at least 3 BC/OC <60y in the family. DNA samples were processed using Agilent's SureSelect Target Enrichment and sequenced on an Illumina MiSeq. Bioinformatic pipeline includes read alignment, variant calling (GATK and VarScan2), and annotation (ANNOVAR and ALAMUT).
Results: In the 49 families sequenced to date we identified 12 truncating variants in *PALB2 (2)*, *ATM (2)*, *BARD1, FANCA, FANCE, FANCI, FANCL, PTEN, SLX4* and *XRCC2*, one missense variant in *TP53*, and 25 variants predicted as probably pathogenic by bioinformatic analysis: 17 (missense) affecting protein function and eight affecting splicing (six missense, one synonymous and one small deletion of a consensus splice site). So far, the gene panel was clinically beneficial for four patients with deleterious variants in *PALB2*, *PTEN*, and *TP53*. Phenotypically, these families had one in situ BC with 1 first degree with BC, both under age 35 (*TP53*), young onset BC with family history of BC and OC (*PTEN*), or an extensive family history of BC (2 *PALB2*).
Conclusions: Multiplex genetic testing of a certain subset of high penetrant genes might be clinically useful for genetic diagnosis of HBOC families.
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PS12.067

Diagnostic yield of a next generation sequencing gene panel in familial cancer analysis

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Background: The current trend in familial cancer diagnostics is the use of NGS gene panels. Potentially, this method could increase the yield of molecular diagnoses. We studied 174 patients suspected of hereditary breast (ovarian) or colorectal cancer, who had been tested negatively for *BRCA1/2* and Lynch syndrome/*APC/MUTYH*, respectively.
Methods: We designed and validated a targeted NGS gene panel with 73 genes associated with familial cancer syndromes. We tested this panel anonymously on all 174 patients. Outcome was interpreted in terms of explanation of the cancer family history. We also compared the test results with the number and types of variants in the panel genes found by WGS in a control group: 498 individuals from the Genome-of-the-Netherlands (GoNL) project.
Results: In 174 patients only one new firm diagnosis was established. In addition, in another 14 cases (8.0%), a pathogenic variant was detected in 6 different autosomal dominant genes traditionally associated with cancer types that did not match the cancer family history. Of these 14 mutations, 10 were actionable. We identified 414 VOUS of which 38 (9.2%) in autosomal dominant genes that were likely pathogenic. In the GoNL controls, 22 (4.4%) pathogenic and 740 VOUS were identified of which 85 (11.4%) were classified as likely pathogenic.
Conclusions: Use of an extended gene panel did not instantly increase the number of molecular diagnoses explaining the cancer family history. Furthermore, many likely pathogenic variants were identified in both our patient and control groups, suggesting that these variants are less relevant in familial cancer diagnostics.

PM12.068

Monoallelic FANCG mutation in a patient with multiple tumours: is FANCG a tumour susceptibility gene?

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We report on the DNA sequencing results we obtained for a 54 year old female patient with suspected familial tumour syndrome. She presented with several tumours (lipoma, myoma, breast cysts) and fibromyalgia. The patient's mother died at the age of 57 of breast cancer. Her maternal grandmother died of a brain tumour. In addition, several family members present with lung cancer (all smokers).
We performed NGS using the TruSight Cancer Panel (Illumina, total of 94 cancer genes). The analysis revealed a heteroallelic frameshift mutation in the FANCG gene: p.A495Qfs*23. No further mutation was detected in the genes covered by the panel, which includes all known breast cancer susceptibility genes and all known Fanconi anemia genes. Unfortunately, no DNA samples of the patient's mother and grandmother are available for segregation analyses.
FANCG is one of the three most commonly mutated genes out of 13 genes coding for proteins active in the same DNA-repair pathway, that may lead to Fanconi anemia (FA) when biallelically mutated (exception: FANCB, X recessive inheritance). FA is a chromosome instability syndrome characterized by childhood anemia, susceptibility to leukemia and cancer.
Monoallelic mutations with increased susceptibility for breast cancer have been reported for only few FA genes: FANCD1 (BRCA2), FANCN (PALB2), FANCJ (BRIP1) and very recently FANCM. We consider the possibility that the FANCG mutation detected in our patient may underlie the familial tumour syndrome in her family and suggest FANCG heterozygosity as a novel breast cancer susceptibility factor.

PS12.069

Characterization of a Fanconi anemia candidate gene

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Fanconi anemia (FA) is a rare autosomal or X-linked recessive disease which displays a heterogeneous phenotype. Besides congenital developmental defects, FA is characterized by bone marrow failure and genomic instability resulting in cancer predisposition. Due to a DNA repair defect the cellular phenotype is characterized by hypersensitivity to DNA-crosslinking agents, leading to increased chromosomal breakage, reduced cell survival and G2 phase arrest in the cell cycle. Currently 16 FA genes (*FANCA, -B, -C, -D1, -D2, -E, -F, -G, -I, -J, -L, -N, -O, -P, -Q* and *-S*), whose products are members of the FA/BRCA pathway, were reported to be causative for FA. Based on the association of corresponding mutations with the loss or retention of FANCD2/FANCI monoubiquitination, these genes are classified as *upstream* or *downstream* FA genes. In one cell line derived from an FA patient we detected a homozygous *single nucleotide exchange* in an FA candidate gene, which is a member of the FA/BRCA pathway but not an FA gene. The mutation results in an amino acid substitution at a conserved position. This cell line exhibits reduced levels of FANCD2 monoubiquitination and FANCD2 foci formation as well as MMC-induced G2 phase arrest in the cell cycle. Complementation with the wild type form of this gene rescues the MMC sensitivity and normal cell cycle progress. In addition, FANCD2 monoubiquitination and FANCD2 foci formation were raised. Currently, we are generating animal and human cell models in order to draw closer connections to the FA/BRCA pathway.

PM12.070

Gene editing of FAAP100 by the CRISPR-Cas9 system

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FAAP100 is a component of the Fanconi anemia (FA) nuclear core complex. Together with FANCL and FANCB it forms a subcomplex, which is necessary for the monoubiquitination of FANCD2 and FANCI and the stability of the core complex. Chicken DT40 cells that are faap100-deficient show a cellular phenotype similar to human FA cells, which includes reduced mitomycin C-induced cell survival and increased chromosomal breakage rates as well as compromised FANCD2 monoubiquitination.
In order to generate an FAAP100-deficient cell line we used the recently discovered RNA-guided CRISPR-Cas9 system. The CRISPR-Cas9 technology for genome engineering is based on the adaptive immune system of bacteria and archaea. This system can be applied to introduce DNA double strand breaks at a target sequence into genomic DNA similar to ZFN nucleases and TALENS. After DSBs have been elicited, the damage can be repaired through error-prone non-homologous end joining (NHEJ) or through error-free homology-directed repair (HDR) if a homologous DNA template is available. In our work, we targeted exon 5 of FAAP100 in U2OS cells. Initial experiments revealed that the CRISPR-Cas9 tool is suitable for tackling FAAP100 in U2OS cells and that stable InDel mutations were introduced through NHEJ. Currently we are trying to isolate single cell clones with a deleterious mutation and to grow an FAAP100 null cell line for further experiments including immunoblots, analysis of chromosomal breakage and cell cycle analysis.

PS12.071

Unusual characteristics in Fanconi anemia subtype FA-Q

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Fanconi anemia (FA) is a rare X-linked or autosomal recessive genetic disorder, characterized by a broad genetic and clinical heterogeneity. Molecular testing is impeded by the presence of at least 16 genes, which are components of the FA/BRCA pathway (FANCA, -B, -C, -D1, -D2, -E, -F, -G, -I, -J, -L, -N, -O, -P, -Q, S). Stringent genotype-phenotype correlations are usually not applicable to these FA subtypes.
A majority of patients belong to the complementation groups FA-A, -C and -G. For several rare subgroups only a few patients have been identified so far. Long term phenotype-genotype data collection of these minor subgroups is essential for a better understanding of the molecular roles of the corresponding proteins as well as for the clinical management of those patients.
Here we report on a 49-year-old patient who belongs to the rare and unusual subgroup FA-Q. Causative are biallelic mutations in the ERCC4/XPF gene. Other mutations in this gene have been associated with Xeroderma pigmentosum, Cockayne syndrome or a progeria syndrome (XF-E). Unlike most individuals with FA our patient never developed bone marrow failure or malignancies, typical features of other FA patients at that age. Interestingly, she shows skin photosensitivity to sunlight, a trait previously not seen in FA patients of other complementation groups except for one of the two previously described FA-Q patients. Hence, sunlight sensitivity in unclassified FA patients may give a hint for their assignment to complementation group FA-Q such that mutations in ERCC4 should be tested preferentially.

PM12.072

Participation of apoptosis-related genes in breast tumorigenesis and their association with clinicopathological features of disease

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Fas and its ligand (FasL) are known to play a crucial role in the genetically controlled mechanism of cell death and disruption of this pathway has been associated with tumorigenesis. The goal of this study is to investigate the association between -1377G/A (rs2234767) and -670A/G (rs1800682) polymorphisms in Fas and single nucleotide polymorphisms -844C/T (rs763110) in FasL in a breast cancer samples. The study group consists of 254 subjects, including 122 patients with breast cancer and 132 healthy controls. DNA extracted from peripheral blood was analyzed by TaqMan real-time PCR and allelic discrimination assay. We have found a significant association between patients with aberrant A allele in genotype for FAS-1377 SNP and larger size of tumors compared to GG genotype carriers (p=0.0356). Moreover patients with FAS-1377A and FASL-844T genotype showed significant association to poorly differentiated breast cancer specimens of higher tumor grade (G2+G3) (p=0.0378 and p=0.0001, respectively). Patients with FAS-1377A and FAS-670G genotype showed significant association to positive expression status of progesterone receptor (p=0.0263 and p=0.0169, respectively). The significant association was observed also between the FAS-670G and FASL-844T genotypes with HER-2 positive status (p= 0.0177 and p= 0.0009, respectively). These data suggest that functional polymorphisms in the death pathway genes FAS and FASL may contribute to the occurrence of breast cancer and modify its biological profile in Slovak population.
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PM12.074

Genetic susceptibility to gastric cancer: replication in Spanish and Portuguese populations of variants identified in the EPIC-EurGast study

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Introduction: In a previous study (EPIC-Eurgast) of association between genetic variation in candidate genes and gastric cancer (GC) risk we observed several associations that required replication in independent populations.
Methods: 96 SNPs from 57 genes that in the EPIC-Eurgast study were found associated with GC or any of its subtypes were genotyped (Fluidigm SNPtype assays) in 328 non-EPIC GC cases and 322 healthy controls from different Spanish regions, as well as 375 GC cases and 709 controls from the North of Portugal. Associations were analyzed using unconditional logistic regression.
Results: Unless otherwise stated, the results indicated are those obtained in the combined analysis of both the Spanish and the Portuguese populations, under the additive model. These results confirmed previously reported associations between GC and PSCA rs2294008 (OR=1,21; 95%CI:1,06-1,40) and MUC1 rs4072037 (OR=0,82; 95%CI:0,72-0,94). Furthermore, associations with GC were replicated in another six genes. DRD4 rs12280580 (OR=0.83;95%CI: 0,72-0,97), VEGFA rs833060 (OR=0,84;95%CI:0,72-0,97), TFF3 rs8133510 (OR=0,75;95%CI:0,61-0,93; AG vs GG) and MIRLET7I rs10877888 (OR=1.29;95%CI:1,00-1,65; recessive model), were associated with GC in general. ABO rs657152 was associated with diffuse GC (OR=1,45;95%CI:1,02-2,06; dominant model) and NQO1 rs7359387 was associated with cardia CG (OR=0,50;95%CI:0,30-0,85).
Conclusions: variation in PSCA and MUC1 also is associated with GC risk in Iberian populations. Variants in genes for cell signalling (DRD4 and VEGFA), mucosa protection (TFF3), region of MIRLET7I (micro RNA let7i), blood group (ABO) and carcinogen metabolism (NQO1), also are associated with GC or its histological/anatomical subtypes in European populations.
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PS12.075

Vitamin D and prostate cancer: a Mendelian randomization study

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Introduction: Decreased 25-hydroxy (25-OH) vitamin D levels have been associated with prostate cancer, but it is unclear whether this association is causal. To examine the hypothesized inverse relationship between vitamin D status and prostate cancer we performed a Mendelian randomization study and studied the association between a common single-nucleotide polymorphism (SNP) strongly associated with 25-OH-vitamin D and prostate cancer.
Material and Methods: Genotypes of the group-specific component gene (GC, rs2282679) were determined in the prospective PROCAGENE study comprising 702 prostate cancer patients with a median follow-up of 82 months. Logistic regression was used to estimate the associations between the genetic variants and biochemical recurrence, development of metastasis and mortality of prostate.
Results: GC genotypes were not associated with biochemical recurrence (HR 0.89, 95% CI 0.70 - 1.13; p = 0.32), development of metastases (HR 1.20, 95% CI 0.88 - 1.63; p = 0.25) or overall survival (HR 1.10; 95% CI 0.84 - 1.43; p = 0.50).
Conclusion: We conclude that a causal role of vitamin D status, reflected by the GC polymorphism, in prostate cancer is unlikely.

PM12.076

The frequency and functional significance of TP53 mutations in the russian patients with de novo DLBCL

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Introduction: *TP53* dysfunction is implicated in lymphomagenesis and disease progression. Information about the frequency and spectrum of *TP53* mutations in the russian pathients with diffuse large B-cell lymphoma (DLBCL) in the current version of the IARC *TP53* Mutation Database R17 is not represented. The goal of this work was to study the frequency, spectrum and functional significance of *TP53* mutations in russian patients with DLBCL.
Material and Methods: At the present time the pilot group of 14 patients were included in the study. Diagnosis was assessed according to the criteria of the WHO classification system. Genomic DNA was isolated from formalin-fixed, paraffin embedded tissue blocks. Direct sequence analysis of gene *TP53* was performed according to the IARC protocol, 2010 update.
Results: In two patients were identified single nucleotide substitutions that are not described in the current version of the PubMed database. All of mutations occurred in the DNA-binding domain of p53. The nonsense mutation Arg196Ter was detected in one patient. Previously it was shown that formation of this premature stop codon might activate the nonsense-mediated RNA decay pathway. The second patient had two missense mutations - Leu130Phe and Arg156Cys. The first of them leads to p53 inactivation according to the analysis of the functional importance of amino acid substitutions using service PolyPhen-2.
Conclusions: We detected *TP53* mutation in 14% cases. The mutational rate in our study is in good agreement with other studies where the frequency of the TP53 mutations in patients with DLBCL ranged mostly from 13 to 23%.

PS12.077

Genome-wide association study of childhood acute lymphoblastic leukemia risk with gender-specificity

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Similar to most cancers, males have a higher risk than females for childhood acute lymphoblastic leukemia (ALL). To gain insight into the genetic basis of this difference, we performed a case-only GWAS to identify gender-specific risk markers among 240 ALL cases recruited at Texas Children's Cancer Center using the Illumina Infinium HumanCoreExome Chip. Results were ranked both by statistical significance and effect size, and then subjected to bioinformatics analysis to evaluate relevant pathways and potential underlying mechanisms. RASSF2 rs4813720 yielded the statistically most significant result for interaction with sex (OR = 0.29; P = 2E-06). Two HLA-DQA1 missense SNPs (rs12722042 and rs12722039) and ADAM28 rs11992342 yielded the largest effect sizes (OR > 14.0; P < 0.05). The HLA-DQA1 SNPs correspond to DQA1*01:07, and their association with risk confirms a previously reported male-specific association with DQA1*01. No sex chromosome variant appeared to be involved in the interaction of risk with sex. While bioinformatic screening of our top hits did not implicate a particular mechanism for male-specific risk, RASSF2 has an estrogen receptor-alpha binding site in its promoter. Additionally, a functional SNP among our top hits (by P value) in (MAGI2 rs798292) is associated with expression of KAT7 in lymphoblastoid cells (P = 2E-08), which is a histone acetyltransferase that represses androgen receptor-mediated transcription. Our results suggest that autosomal variants are likely to underlie the sex differential in childhood ALL risk. Our approach and results provide a foundation for further studies to fully characterize the sex differential in childhood ALL risk.

PM12.078

Spectrum of mutations identified in a 25-gene hereditary cancer panel for patients with breast cancer

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Introduction: Advancements in next generation sequencing allow patients with a personal and/or family history of cancer that may not be suggestive of one cancer syndrome to be tested for mutations in multiple cancer-predisposing genes simultaneously. The focus of this analysis was to determine the spectrum of gene mutations observed in patients with a personal history

of breast cancer.
Methods: A commercial laboratory database was queried for patients with a personal diagnosis of breast cancer who underwent testing with a 25-gene hereditary cancer panel from September 2013 through November 2014. The panel includes *BRCA1, BRCA2, TP53, PTEN, MLH1, MSH2, MSH6, PMS2, EPCAM, APC, BMPR1A, CDH1, CDKN2A, MUTYH, SMAD4, STK11 CHEK2, PALB2, ATM, NBN, BARD1, BRIP1, CDK4, RAD51C* and *RAD51D*. All patient data regarding clinical history was obtained by health care provider report on the test requisition forms.
Results: A total of 17,142 patients with a personal history of breast cancer were identified. In this cohort, 9.8% (n=1,685) of patients were positive for at least 1 pathogenic mutation, 49.9% of which were in *BRCA1/2*. The 51.1% of patients with mutations in genes other than *BRCA1/2* included 151 patients with mutations in genes not associated with breast cancer. 45 patients were identified with mutations in more than one gene.
Conclusions: Testing patients using a 25-gene panel provided a 104.5% increase in mutations identified over *BRCA1/2* testing alone. Panel testing also allowed patients with mutations in genes not associated with breast cancer or with multiple mutations to make more appropriate medical management decisions.

PS12.079

Identification of gains and losses of genomic regions characteristic for head and neck cancers of oral cavity and oropharynx/hypopharynx.

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Introduction
Copy number variations of large genomic regions are an important mechanism implicated in the development of head and neck cancer however for most changes their exact role is not well understood. It was the aim of this study to find possible associations between gains/losses of genomic regions and clinically distinct subgroups of head and neck cancer.
Methods
Array CGH analysis was performed on DNA samples from 64 patients with cancer in oral cavity oropharynx or hypopharynx. Overlapping genomic regions created from gains and losses were used for statistical analysis.
Results
Following regions were overrepresented: in tumors with stage I,II a gain of 2.98 Mb on 6p21.2-p11, a gain of 7.4 Mb on 8q11.1-q11.23; in tumors with grade I histology a gain of 1.1 Mb on 8q24.13, a loss of a large part of chromosome 3p, a loss of a 1.24 Mb on 6q14.3, a loss of 32 Mb telomeric region (8p23.3); in cases with affected lymph nodes a gain of 0.75 Mb on 3q24, a gain of 0.9 Mb on 3q26.32-q26.33; in cases with unaffected lymph nodes a gain of 1.1 Mb on 8q23.3; in patients not treated with surgery a gain of 12.2 Mb on 7q21.3-q22.3 and a gain of 0.33 Mb on 20q11.22.
Conclusions
Our analysis identified several genomic regions of interest which appear to be associated with various clinically distinct subgroups of head and neck cancer. They represent a potentially important source of biomarkers useful for clinical management of head and neck cancer.
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PM12.080

Whole exome sequencing identifies novel variants for head and neck squamous cell carcinoma

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Introduction: The head and neck squamous cell carcinoma (HNSCC) is ranking as the fifth most common cancer worldwide and it is also the second leading cause of cancer deaths in the South-East Asia region, including Taiwan. The major risk factors associated with HNSCC are smoking, alcohol consumption, and human papillomavirus subtype 16 (HPV-16) infection. However, most of the HNSCC patients in Taiwan were HPV-16 infection negative. This implicates that genetic variants underlying the HNSCC in Taiwan might be different from other populations. To obtain a comprehensive overview of genetic alternations underlying HNSCC, we applied whole-exome-sequencing on HNSCC patients.
Methods: Nine pairs of tumor and adjacent normal tissue samples with stage III-IV HNSCC were obtained from the Tissue Bank of the TPEVGH. DNA were captured using SureSelect Human All Exon kit and Illumina Paired-End Sequencing Library Prep, then were sequenced by Illumina Genome Analyzer IIx platform.
Results: In total, 1788 novel variants, including 676 missense, 146 synonymous, 61 nonsense, and 7 splicing site variants, were identified (with an average 199 variants per patient, range 51-532 variants). Genetic variants on ninety-nine genes were recurrently found in maximally two patients. Five out of nine HNSCC patients carried the TP53 novel variants. Most of the variants are located on the PI3K, MAPK, EGFR, and NOTCH pathways, while some of them are oncogenes, tumor suppressor genes, and DNA repair genes.
Conclusion: Using exome sequencing, we identified several candidate genes for HNSCC predisposition. Their contribution to HNSCC will be further investigated by using larger cohort of patients with HNSCC.

PS12.081

NOVEL CHEK2 LARGE GENOMIC REARRANGEMENTS IDENTIFIED IN GREEK BREAST CANCER PATIENTS

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Although certain germline missense and loss-of-function CHEK2 mutations have been reported, Large Genomic Rearrangements (LGRs) seem to be quite rare genomic events. To date, only two LGRs have been reported, a 5.6 kb deletion encompassing gene's exons 9 and 10, which is a founder Czech mutation, and a duplication encompassing gene's exons 8 to 14.
The present study aimed to define the contribution of CHEK2 LGRs in Greek breast cancer patients.
Initially, 2355 breast cancer patients (mean age of onset 54.6 years) were genotyped for the Czech LGR by diagnostic PCR. No individuals of Greek descent were found to carry the particular deletion.
Subsequently, three novel LGRs were detected by BROCA panel and were confirmed by MLPA. These included: a ~600bp deletion including the 5' UTR region, a ~1100bp deletion of exons 2 and 3 and a ~7.5Kb deletion including exon 6. The genomic breakpoints of the latter were determined (g.30219_37783del7565, p.D308fsX0) and subsequently analyzed by a custom-designed diagnostic PCR. The deletion was detected in 0.22% (5/2355) of breast cancer cases, indicating a possible Greek founder effect. Three carriers were diagnosed with early onset breast cancer, while all of them had at least one additional family member diagnosed with breast cancer. None of the 1163 controls carried the aforementioned deletion. Characterization and screening of the two other LGRs is in progress.
The present study highlights the existence of rare genomic events in breast cancer predisposing genes which can explain a small -but significant- proportion of high risk families.

PM12.082

Prevention of hereditary breast cancer by personalized optimization of body Se, Zn, Fe levels using diet supplements

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Hereditary predisposition to breast cancer (HBC) can be diagnosed in ~1.5 mio. of women in Poland including ~100 000 carriers of BRCA1mutations. Up to now, the critical preventive options for above females give strong adverse effects (preventive adnexectomy and mastectomy, tamoxifen). Therefore, the main goal of herein project is validation of results from pilot studies indicating that risk of breast cancers can be reduced significantly (even 10 times) by personally tailored optimization of Se, Zn and Fe levels using diet supplements.
The major tasks will include:
I. Elaboration of diagnostic "DNA test basic for chemoprevention of breast cancers." (10 candidate changes; sera collected before breast cancer detection in 150 cases; 300 matched controls).
II. Hereditary breast cancer risk reduction by providing Se, Zn, Fe diet supplements in amounts to achieve optimal serum levels individualizing them also depending on DNA changes. (2000 females with BRCA1 mutation, 12 000 unaffected women with other forms of HBC randomly selected for studied and control groups; expected results - patents owned by Read-Gene SA and publications on new method of HBC prevention.
III. Zn, Fe diet supplements - large scale production, studies of their stability

and delivery for clinical trial.
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PS12.083

Increased risk of male cancer and identification of a potential prostate cancer cluster region in *BRCA2*

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Background The risk of cancer in men from BRCA families is relevant to define to motivate genetic testing and optimize recommendations for surveillance. In a cohort of 301 Danish families with mutations in *BRCA1* and *BRCA2* we assessed the risk of cancer in male mutation carriers and first-degree relatives in relation to a sex-matched and birth-matched control population.
Results Men in *BRCA1* families were not at increased risk of cancer, except for a trend for prostate cancer before age 65, whereas men in *BRCA2* families were at increased risk of male breast cancer and prostate cancer. Male *BRCA2* mutation carriers were at a cumulative risk of 11% for breast cancer 19% for prostate cancer. Male breast cancers developed at a mean age of 59 years and were typically ER/PR positive ductal carcinomas. Prostate cancer developed with a HR of 4.0 (p<0.001) in mutation carriers and a HR 3.3 (p=0.001) in first-degree relatives. The *BRCA2*-associated prostate cancers developed at a mean age of 68 years and were frequently high-grade with Gleason scores ≥8 in 22% of the tumors. Genotype - phenotype correlations were identified with a HR of 9.7 (p≤0.001) for men with mutations in a region of *BRCA2* defined by nucleotides 6373-6492.
Conclusion Male mutation carriers and first-degree relatives in *BRCA2* families are at an increased risk of breast cancer and prostate cancer with a potential prostate cancer cluster region within the *BRCA2* gene.
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PM12.084

Identification of rare CDH1 non-coding variants in Hereditary Diffuse Gastric Cancer

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Introduction
Hereditary diffuse gastric cancer (HDGC) is a rare, severe, highly penetrant and difficult to diagnose syndrome. Forty-five percent of HDGC-families are associated with germline CDH1/E-cadherin coding alterations.1-3 Other susceptibility genes were recently implicated in ~5% of CDH1-negative families.4 Of the 50% that remain without molecular diagnosis, 2/3 present germline monoallelic CDH1 downregulation5,6 and >90% display loss of E-cadherin expression in tumours.7 HDGC families lacking CDH1-coding mutations may harbour causative germline alterations in non-coding regions.
Material and Methods
The entire CDH1 locus was sequenced from the germline DNA of 90 HDGC probands without mutations in classically screened regions. Bioinformatics criteria were used to prioritize novel NCVs to be evaluated from a biological standpoint: 1) chromatin status from the adjoining region; 2) occurrence in putatively transcribed regions; 3) conservation level; and 4) predicted creation/deletion of repressors/enhancers consensus sequences.
Results and Discussion
Thirty rare heterozygous germline CDH1 NCVs were sorted-out as putatively disease causative for 25% of CDH1-negative probands. NCVs were clustered in silico suggesting the existence of two different pathways impacting gene expression: 1) promoter/enhancer-related variants that create/eliminate binding sites for CDH1 expression repressors/enhancers; and 2) transcription-related variants that may impair the normal CDH1 locus expression pattern. This hypothesis will be biologically addressed in order to confirm NCVs impact in CDH1 expression. Any pathogenic alteration will warrant a complete redefinition of the screening methodology currently applied to HDGC families, providing simultaneously important insights for the understanding of CDH1 expression regulation.

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PS12.085

A cancer susceptibility hotspot in HLA class I region

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The HLA region is well recognized as the regulator of immune response, but is less known for its non-HLA gene content. Besides having the highest gene density in the genome, the HLA region also has remarkable gene diversity. A particular subregion in the HLA class I region contains a number of candidate cancer susceptibility genes and a large number of ncRNA genes. Using disease association databases, we screened SNPs from the overlapping genes CCHCR1/TCF19/POU5F1 and flanking regions (chr6:30.9-31 .4Mb) correlated with cancer susceptibility, and annotated them to assess their causality. At the statistical significance threshold of 0.0001, there were independent associations (rs6457327, rs1634718, rs130067, rs2596503, rs3130544, rs7750641) with lung/breast/cervical/prostate cancers, non-Hodgkin lymphoma, and glioma. The lung cancer-associated SNPs were not in LD (r2<0.50) with the primary lung cancer risk marker BAG6 rs3117582. Each one of these SNPs had a RegulomeDB score <2b and/or CADD C-score >2.5, suggesting high functionality, although none of the cancer-associated SNPs in the region were deleterious to protein function. When their statistically similar (r2>0.8) SNP sets were examined, they also had high functionality scores (C-scores up to 15.8; RegulomeDB scores up to 1f). Most of these SNPs were eQTLs for ncRNAs in this subregion (linc00243 which derives from IER3; HCG22 which is expressed exclusively in lymphoid tissues, lung, breast, prostate, brain and few other tissues). These data suggest that the subregion of HLA class I region examined in this survey contains multiple independent cancer susceptibility risk for multiple cancer types, which may act via modifying the activity of ncRNA content.

PM12.086

Determination of the JAK2 V617F mutation in thrombosis patients

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Introduction: Janus kinase 2 (JAK2) gene mutation causes uncontrolled myeloproliferation independent of cytokines and abnormal formation of endogenous erythroid colony. This mutation was found in myeloproliferative disorders (MPDs) especially in polycythemia vera (PV) and essential thrombocythemia (ET). In addition, MPDs represent a risk factor for development of thrombosis that is a major cause of morbidity and mortality in patients.
Materials and Methods: We aimed to consider the correlation between JAK2 mutation and thrombosis. Thirty-nine patients who had clinical diagnosis as thrombocytosis and Budd-Chiari syndrome from 2010 to 2014 were collected to determine JAK2 V617F mutation. DNA from all specimens were amplified and detected the presence of JAK2 V617F mutation by AS-PCR.
Results: In this study, we demonstrated JAK2 V617F mutation in both patients who had clinical diagnosis as thrombocytosis and Budd-Chiari syndrome. We found that 11 of 37 (29.7%) thrombocytosis patients had JAK2 V617F mutation. Moreover, one of two patients who had represent as Budd-Chiari syndrome was detected JAK2 V617F mutation.
Conclusions: JAK2 V617F mutation has associated with thrombosis. However, further study in large series is needed to support. Determination of the JAK2 V617F mutation may be useful for screening latent or occult MPDs patients who have occurrence of thrombosis to adjust the appropriate treatment for good patient outcome.

PS12.087

Revisiting the Li-Fraumeni syndrome from 415 TP53 mutation carriers

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Li-Fraumeni syndrome (LFS) is a remarkable cancer predisposition characterised by extensive clinical heterogeneity. We performed, in the context of a national study, a clinical update from the mutation carriers that we identified over the last 20 years. From 1730 patients suggestive of LFS, we identified 415 mutation carriers in 214 families harbouring 133 distinct *TP53* alterations. The 322 affected carriers developed 552 tumours and 43% had developed multiple malignancies. The mean age of first tumour onset was 24.9 years, 41% being affected by age 18. In childhood, the LFS tumour spectrum was characterised by osteosarcomas, adrenocortical carcinomas (ACC), central nervous system tumours, and soft-tissue sarcomas (STS) observed in 30, 27, 26 and 23% of the patients, respectively. In adults, the presentation was characterised by the predominance of breast carcinomas observed in 79% of the females, and STS observed in 27% of the patients. The *TP53* mutation detection rate in children with ACC or choroid plexus carcinomas, and in females with breast cancer before 31, without additional features indicative of LFS, was 45%, 42% and 6%, respectively. The mean age of tumour onset was statistically different ($p<0.05$) between carriers harbouring dominant-negative missense mutations (21.3 years) and those with all types of loss of function mutations (28.5 years) or genomic rearrangements (35.8 years). Affected children, except those with ACC, harboured mostly dominant-negative missense mutations. The clinical gradient of the germline *TP53* mutations suggests that it might be appropriate to stratify the clinical management of LFS according to the class of the mutation.

PM12.088

Comparison of Real-Time PCR, fluorescent in situ hybridization and Immunohistochemistry for detection of MDM2 gene amplification in Well-Differentiated and Dedifferentiated Liposarcoma about 15 cases

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Introduction
Liposarcomas are a heterogeneous group of malignant adipocytic neoplasms that consist of 3 distinct clinicopathological entities: well-differentiated/dedifferentiated liposarcoma, myxoid liposarcoma, and pleomorphic liposarcoma. Dedifferentiated liposarcoma (DDLPS) results from the progression of a well-differentiated liposarcoma (WDLPS) to a nonlipogenic sarcoma of variable histologic grades and morphologic patterns that acquires metastatic potential.
Our objective is to a correlate between Real-Time PCR (RT-PCR), fluorescent in situ hybridization (FISH) and Immunohistochemistry (IHC) for detection of MDM2 gene amplification in WDLPS and DDLPS.
Methods
15 cases of liposarcoma were selected from the Pathological Anatomy service to make this correlation. Diagnosis is based on a standard histology. Immunohistochemical confirmation was carried out by the antibodies anti-MDM2 and anti-CDK4. The amplification of MDM2 was performed by FISH and RT-PCR.
Results
The average age of patients is 54 years, ranging from 27 to 75 years with a slight female predominance.
8 cases of tumors are diagnosed WDLPS and 7 other cases DDLPS. These tumors are characterized by a heterogeneous aspect of malignant cells and positivity of anti-MDM2 and anti-CDK4 antibodies. MDM2 amplification by FISH was found in 6 of 15 liposarcoma cases ((3/8) of WDLPS and (3/7) of DDLPS) and in 7 cases by RT-PCR ((3/8) of WDLPS and (4/7) of DDLPS) something which shows more than 85% concordance between the two techniques knowing the sensitivity of FISH is higher than that of the RT-PCR.
Conclusions
MDM2 amplification has been shown to have high sensitivity in characterizing WDLPS and DDLPS.
Differences in MDM2 amplification profiles among liposarcomas could help further define and predict progression to high-grade neoplasia.

PS12.089

Rapid aneuploidy screening of plasma DNA samples from cancer patients using a modified FAST-SeqS approach

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Introduction
Recent progress in the analysis of cell-free DNA fragments (cell-free circulating tumor DNA, ctDNA) now allows monitoring of tumor genomes by non-invasive means. However, previous studies with plasma DNA from patients with cancer demonstrated highly variable allele frequencies of ctDNA. The comprehensive analysis of tumor genomes is greatly facilitated when plasma DNA has increased amounts of ctDNA. Therefore, a fast and cost-effective pre-screening method to identify such plasma samples without previous knowledge about alterations in the respective tumor genome could assist in the selection of samples suitable for further extensive qualitative analysis.
Methods
To address this, we adapted the recently described FAST-SeqS method, which was originally established as a simple and effective, non-invasive screening method for fetal aneuploidy from maternal blood.
Results
We show that our modified FAST-SeqS method (mFAST-SeqS) can be used as a pre-screening tool for an estimation of the ctDNA percentage. Plasma samples with an mFAST-SeqS z-score above 5 showed highly concordant results compared to copy number profiles obtained from our previously described plasma-Seq approach. Therefore, mFAST-SeqS revealed a general overview about the aneuploidy status of the tumor genome on the resolution of chromosome arms.
Conclusion
Advantages of this approach include that no prior knowledge about the genetic composition of tumor samples is necessary in order to identify between plasma DNA samples with more than 10% of ctDNA content, and the speed and cost-effectiveness of the assay.

PM12.090

Pharmacogenomic study of non-small cell lung cancer

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Background: Lung cancer is a leading cause of death. Non-small cell lung cancer (NSCLC) accounts for approximately 85% of all cases. Platinum based therapy is used as standard treatment for patients with NSCLC. The epidermal growth factor receptor (EGFR) has been shown to play an important role in the growth and survivor of many solid tumors, including NSCLC. Gefitinib and erlotinib are EGFR tyrosine kinase inhibitors (EGFR-TKI) that blocks the signal transduction pathways implicated in the proliferation and survival of cancer cells. Little is known about other pharmacogenetic variants in NSCLC.
Methods: We performed DNA analysis on 19 patients with NSCLC. Tumor sample DNA was extracted from formalin-fixed paraffin-embedded samples. The samples were NGS sequenced using Illumina cancer panel (96 genes and 256 variants) by MiSeq instrument. The pharmacogenetic significance of our variants was evaluated using the information from PharmGKB database.
Results: Pharmacogenetic variants were found in different genes: ERCC2 (63% of the samples), TP53 (74%), XPC (68%). These variants define NSCLC as sensitive to treatment with the platinum based therapy. Another variant in EGFR gene (EGFR R497K) sensitive to EGFR-TKI was found in 47% of the samples by NGS sequencing. These variants can't be detected by the current IVD labelled real time PCR assays for NSCLC target therapy.
Conclusion: Our data shows that next generation sequencing tests reveals a new useful information for the personalized therapy of NSCLC.

PS12.091

HLA Region Contains Multiple Lung Cancer Susceptibility Genes

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The major histocompatibility complex (MHC) was strongly implicated in the development of lung cancers in animal experiments. Lung cancers show the highest number of cancer associations with human MHC (HLA) region. For a systematic analysis, we extracted 73 SNPs located within chromosome 6: 29 to 33Mb and associated with lung cancer from the GWAS catalog, dbGAP, and GRASP database (P<5E-04), of which 59 exceeded the genome-wide statistical significance threshold (P < 5E-08). Only three of those were listed in the GWAS catalog (rs3117582, rs3817963, rs2395185), with BAG6 rs3117582 being the most consistently observed. Associations were with squamous

cell and adenocarcinoma, as well as lung cancers in never-smoked women. These SNPs also show associations with Hodgkin lymphoma, breast, and prostate cancers. Three SNPs occur as somatic mutations in breast and colon cancers (rs7750641, rs3749971, rs3134942). Two SNPs (rs3117582, rs1150752) map into CgG islands, one (rs9276472) is within 500bp of a microRNA sequence (MIR3135B), three in non-coding RNA genes, and two are deleterious missense variants (rs9262143, rs7775397) in KIAA1949 and C6orf10. Only one SNP was in an HLA gene (rs2187668, HLA-DQA1, non-coding) and none were in a sequence encoding peptide-binding region of HLA molecules. However, 35 of the 73 SNPs scored the highest gene regulatory function in RegulomeDB analysis. Most notably, rs3117582 and its six statistically indistinguishable (r2 = 1) and 41 statistically similar (r2 > 0.8) SNPs had high levels of functionality. These results strongly suggest that the HLA region contains multiple lung cancer susceptibility markers acting via more than one and non-immune-related mechanism.

PM12.092

Genomewide Copy number variation analysis and Targeted Next Generation Sequencing in suspected Lynch syndrome families reveals novel potential causative candidate genes

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Introduction: In up to 50% of suspected Lynch syndrome (LS) families with typical signs of mismatch repair (MMR) defect in tumor tissue, no germline mutation in the MMR genes can be detected. Loss-of-function copy number variants (CNVs) in these patients might give a hint to yet unidentified genes responsible for LS.
Methods: Genomic DNA from 81 unrelated mutation-negative patients from the German HNPCC Consortium and four patients from the Netherlands was genotyped. Putative CNVs were identified and filtered according to stringent criteria to select rare, non-polymorphic deletions and duplications. Candidate genes in validated CNVs were screened for germline point mutations by a targeted NGS approach in 44 patients.
Results: In total, 30 deletions and 18 duplications encompassing 71 protein-coding genes were found in 35 patients. Five of these genes are promising candidate genes that are highly expressed in normal colorectal tissue. Three of these genes are involved in cell adhesion, cell development and transformation, cell cycle checkpoint regulation, and cell volume or polarity control. One is involved in double strand break repair and recombination and the last one possesses DNA helicase activity. Analysis of NGS data is still ongoing.
Conclusion: By applying stringent filter criteria we identified a group of rare, non-recurrent loss-of-function CNVs which might contain novel predisposing genes for LS. The ongoing NGS analysis will reveal whether there are also point mutations in one of these genes confirming a possible causative effect for LS.
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PS12.093

Unexplained hereditary hypermethylation of the *MSH2* promoter

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A 43-years old male was diagnosed with two primary colorectal cancers (CRC). The family fulfilled the Amsterdam criteria, with a father and paternal grandmother with CRC at age 57 and 50, respectively. A sister was diagnosed with a colorectal adenoma at age 42.
The CRCs of both our index and his father were MSI-high with lack of nuclear staining of MSH2. Neither a germline mutation in *MSH2* or *MSH6* nor a copy number alteration in *EPCAM* and the intergenic region between *EPCAM* and *MSH2* was detected. In the 3'UTR of *EPCAM,* about 280 nt upstream of the poly-adenylation signal, a heterozygous variant was present: c.*136C>G. Hypermethylation of the *MSH2* promoter was found in both tumors and normal colon tissue, but not in peripheral blood or oral mucosa cells of our index patient, mimicking the situation in patients with a 3'end *EPCAM* deletion.
The heterozygous *EPCAM* variant cosegregated
with hypermethylation of the *MSH2* promoter in his father and sister. A Lynch-like surveillance regimen was advised for all first-degree relatives.
Cosegregation of the *EPCAM* variant c.*136C>G with *MSH2* promoter methylation in the tumors indicate that an aberration in the *EPCAM-MSH2* locus other than a deletion of the polyadenylation signal of *EPCAM* can induce hypermethylation of the *MSH2* promoter. Whether there is a causal relation between this variant and hypermethylation of the *MSH2* promoter is currently unclear.

PM12.094

An unusual case study of Lynch syndrome solved through Next Generation sequencing

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Lynch syndrome caused by inherited *MSH6* mutations is characterised by an increased cancer risk, predominantly of the bowel and endometrium. This case study describes a cluster of three endometrial cancers in first-degree relatives, with the proband diagnosed at 60 years. Her mother and sister were diagnosed at 59 and 74 years, respectively. The proband's tumour showed loss of *MSH2* and *MSH6* protein immunostaining, but no mutations in either gene were identified by dHPLC and MLPA testing methods in 2008. Colonoscopy screening was initiated for the proband and close family members according to Lynch syndrome guidelines. In 2014, *MSH2* and *MSH6* gene testing was repeated using Next Generation sequencing and a heterozygous duplication of a single nucleotide in exon 5 of *MSH6* was identified which was verified by fluorescent sequence analysis. This finding confirmed the diagnosis of Lynch syndrome in the proband and the need for bowel screening in this family. The result also enabled predictive gene testing in relatives and the exclusion from risk of some individuals found not to carry the familial mutation.
This case study highlights the importance of Lynch syndrome testing in families with endometrial cancer that do not meet clinical diagnostic criteria. It also shows 1) the utility of multi-gene testing following aberrant immunostaining results and 2) how updated genetic testing techniques can detect pathogenic mutations previously missed by older methods. The role of the Clinical Genetics professional in conveying new personal risk information derived from the use of updated technology is also discussed.

PS12.095

Assessment of clinical history, immunhistochemical phenotype, and germline DNA analysis in detection of LS-related PMS2 gene mutations in at risk individuals from Ontario/Quebec, Canada.

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Lynch Syndrome (LS) is characterized by mutations in the DNA mismatch repair genes MSH2, MLH1, MSH6, and PMS2. However, the identification of PMS2 specific mutations is complicated by the presence of PMS2 pseudogenes, along with the allelic diversity of the 3' region of PMS2, a complexity which has probably led to under-reporting PMS2 gene mutations as a cause of LS. Acting as a referral centre serving a population of >2m. people we have developed a technique to identify deleterious PMS2 gene mutations by combining MLPA analysis with SNP-specific probes, along with methodology, adapted from Clendenning et al. (Human Mutation, 2006), utilizing genomic long-range PCR enabling sequence analysis of both PMS2 and PMS2CL.
Over the past two years 85 patients were referred to this centre for PMS2 analysis because of a personal and family cancer history, and/or mis-match repair protein immunohistochemical (IHC) tumour analysis suggestive of LS. IHC findings were reviewed where available, along with tumour DNA for microsatellite instability, and leukocyte-derived germline DNA was examined for PMS2 gene mutations.
24 of the 85 referred patients demonstrated an isolated IHC loss of PMS2, 14 of the 24 (58%) proved to be PMS2 mutation positive, with 2 of the 24 demonstrating an MLH1 mutation. In the remaining 61 patients (primarily showing MLH1/PMS2 loss by IHC) no PMS2 gene mutations were identified. We propose that it is reasonable to limit PMS2 gene analysis to patients considered to be at risk of LS to those individuals who demonstrate isolated IHC loss of PMS2 protein.

PM12.096

Uterine cancer in Lynch syndrome: clinico-pathological and molecular features

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Background: Lynch Syndrome (LS) arises from a germline mutation in MMR genes and predisposes to colorectal and uterine cancers (UC). The aim of this work was to deeply characterize the UC in LS. **Methods:** 29 LS-UC and 38 sporadic cancer for comparison were studied for clinicopathological features, MMR IHC, MSI and MLH1 methylation. **Results:** The mean age at the UC diagnosis of LS patients was 46 years respect to patient with sporadic UC (55 years, p=0.007). BMI of LS patients was 24 (normal weight) respect to the patients with sporadic cancer (BMI=27,3; p=0.04). UC arose as first manifestation of the pathology in 34,5% of LS patients. Corpus of the uterus was the prevalent site of onset, while 3 LS-UC arose in the Lower Uterine Segment (LUS) and 4 in the cervical canal. Most of the tumors showed an endometrioid histotype. Abundant tumor-infiltrating lymphocytes (TIL) and peritumoral lymphocytes (PL) were observed in LS-UC in comparison to the sporadic ones. IHC loss of at least one MMR protein was observed in 27 cases (93%), including 19 cases with MSH2 and/or MSH6 loss and 8 cases with MLH1 and PMS2 loss. MSI was detected in 27 cases (93%). Interestingly, 4 LS-UC presented also MLH1 promoter methylation. **Conclusions:** This study demonstrates that the evaluation of these clinico-pathological and molecular features is an efficient strategy to identify LS and to refer those patients to the genetic counseling.

PS12.097

Developing and implementing gene-specific clinical management guidelines for Lynch syndrome

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Background: Lynch syndrome is genetically heterogeneous, caused by mutations in the mismatch repair (MMR) genes: MLH1, MSH2 (including EPCAM-deletion mediated MSH2 hypermethylation), MSH6 and PMS2. It is characterised by predisposition to colorectal (CRC), endometrial and other cancers. Cancer surveillance and risk-reduction strategies have been introduced on the basis of cancer risks in MLH1 and MSH2 mutation carriers. However, emerging data suggest the cancer spectrum and penetrance may differ substantially between the MMR genes.
Objectives: To review cancer penetrance and evidence for risk-reducing interventions in MMR mutation carriers, and to provide management recommendations for carriers based on individualised cancer risks.
Methods: A systematic literature review was conducted to identify relevant studies meeting inclusion and quality criteria. Using these data management recommendations were then developed and implemented following a consensus meeting.
Results: Available data supports gene-specific management protocols for the MMR genes. We recommend colonoscopy at 18-monthly intervals starting at 25yrs for MLH1 and MSH2 carriers and 30yrs for MSH6 and PMS2 carriers. This is based on the reduced and later age-dependent CRC penetrance for MSH6 and PMS2 carriers. We recommend female MLH1 and MSH2 carriers consider risk-reducing hysterectomy and bilateral-salpingo-oophorectomy (BSO) from 40yrs, but available data does not support recommendation of BSO in premenopausal MSH6 and PMS2 carriers.
Conclusion: We have implemented gene-specific protocols for MMR mutation carriers at the Royal Marsden. The protocols, accompanying FAQs and background document are freely available at www.icr.ac.uk/protocols (protocols 9-12).
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PM12.098

Prognostic significance of cytogenetic and molecular-genetic changes in patients with MDS

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Introduction: The myelodysplastic syndromes are a group of hematopoietic disorders characterized by clonal hematopoiesis, impaired differentiation, peripheral-blood cytopenias, and a risk of progression to acute myeloid leukemia. Conventional cytogenetic analysis (CCA) is one of many prognostic factors included in International Prognostic Scoring System (IPSS).
Patients and Methods: We investigated 804 patients with primary MDS in years 2003-2014. The CCA was performed on bone marrow samples using short time cultivation. Interphase FISH was carried out using DNA probes designed to detect 5q, 7q, 11q, 12p, 17p and 20q deletions, trisomy 8, loss Y (MetaSystems Germany, Abbott Molecular).
Results: We cultivated 94,5 % of samples successfully. Chromosomal aberrations were detected in 23 % of patients using CCA and I-FISH. CCA revealed chromosomal aberrations in 9 % of patients with negative I-FISH results. For statistical analysis of our data we followed The New Comprehensive Cytogenetic Scoring System of chromosomal aberrations in MDS defined by Schanz *et al.* (2012) and we classified patients according to chromosomal abnormalities into five prognostic subgroups: *very good* - 3,0 %, *good* - 80,9 %, *intermediate* - 5,8 %, *poor* - 3,7 % and *very poor* - 6,6 %.
Conclusion: Our comparison with New Comprehensive Cytogenetic Scoring System defined by Schanz *et al.* (2012) will be discussed in the poster. However we were able to classify 757 of our patients into this scoring system. According to our results it is important to combine both cytogenetic methods (CCA and I-FISH) to correct classification of patients into prognostic subgroups and suggest appropriate therapy.

PS12.099

The analysis of somatic mutations in melanoma patients using hybridization with diagnostic biochips

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Melanoma is one of the most aggressive cancers. Target inhibitors are being developed for the treatment of cancer patients with particular tumor genotype. A diagnostic biochip has been developed which allowed analyzing 40 somatic mutations in *BRAF*, *KIT*, *NRAS*, *MEK1/2*, *GNAQ* and *GNA11* genes potentially relevant to existing and emerging targeted therapies in melanoma. The technique of LNA-blocking PCR was used for specific amplification of mutant DNA in a large excess of wild-type DNA. The amplified fragments were labeled via incorporation of fluorescently labeled nucleotide during the second round of PCR and were hybridized with specific oligonucleotides immobilized on a biochip. The assay was tested using cell lines, fresh-frozen tissue, and formalin-fixed paraffin embedded tissue. The clinical samples from 52 patients with melanoma of skin were analyzed. Mutation in *BRAF*, *NRAS*, *KIT* genes were detected in 52%, 15% and 3% of patients, respectively. The validation was done using Sanger sequencing. This approach was able to detect approximately 1% of mutated alleles in wild-type DNA background.
We consider that the biochip-based assay is a reliable and inexpensive method for the identification of melanoma patients, who may respond to a specific target therapy.
This work was supported by grant of the Russian Science Foundation (grant # 14-35-00107) and the co-financing provided by the Federal Agency for Scientific Organizations.

PM12.100

Genetics of inherited cutaneous melanoma in Latvia: functional and next-generation sequencing approach

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Introduction: Approximately 10% of cutaneous melanomas occur in the familial setting. Hereditary melanoma is mainly caused by germline mutations in the *CDKN2A* gene (in approximately 39% of all families) and rarely in *CDK4*, *BAP1*, *POT1*, *MITF*, and *TERT* genes. However, in more than half of the families the inherited mutations causing melanoma are still unknown. In Latvia melanoma predisposition is mainly associated with R24H mutation in the *CDK4* gene and recently the first family with *CDKN2A/p14ARF* promoter deletion c.– 20677_–20682delGTACGC was found. Although, in the majority of families (19/23 (83%)) the inherited gene mutations causing melanoma are not found. The aim of this study was to analyse the association of *CDKN2A/p14ARF* promoter deletion with melanoma and to test its functionality, as well as, to analyse Latvian melanoma families for mutations in newly characterized melanoma susceptibility genes using next-generation sequencing.
Materials and Methods: *CDKN2A/p14ARF* promoter region with deletion was genotyped on an ABI 3100 Sequencer. Two sided Fisher's exact test was applied for statistical analysis. Luciferase expression system was used for

deletion's functionality assessment. Agilent SureDesign tool was used to create HaloPlex custom design panel for next-generation sequencing on the Ion Torrent™ system.
Results: *CDKN2A/p14ARF* deletion c.-20677_-20682delGTACGC was found in 7 of 211 melanomas patients and 2 of 326 controls (OR=6.35, 95% CI 1.34-30.22, p=0.017). Deletion's functionality and next-generation sequencing results using HaloPlex platform that includes six full size (*BAP1*, *TERT*, *POT1*, *CDKN2A*, *CDK4*, *MC1R*) and 60 SNPs from 15 other melanoma susceptibility genes will be discussed.

PS12.101
Severity score for multiple endocrine neoplasia

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Multiple endocrine neoplasia (MEN) is autosomal dominantly inherited tumor syndrome which is characterized by occurrence of various endocrine and non-endocrine tumors. Clinical features and severity of each manifestation varies among patients even in the same family. As involvement of many specialists of different specialty is necessary for comprehensive clinical care of patients with MEN, parameters to objectively evaluate patient's clinical severity will be useful. For this purpose, members of the "MEN consortium of Japan" prepared a "Severity score chart for MEN1 and MEN2" in trial. In this chart, evaluation of each patient is performed with three steps. In Step 1, absence or presence of each disorder and degree of burden is evaluated by five-level rating scale (0, absence of the disease; 4, the most severe). Degree of burden is judged based on Karnofsky Performance Status Scale (Karnofsky and Burchenal, 1949). Then in Step 2, absence or presence of distant metastasis of the tumor is scored and in Step 3, severity grade of the patient is judged. Presymptomatic subjects who have pathogenic mutation but no apparent diseases are categorized as Grade I and the most severe case is categorized as Grade VI. Although current trial model solely evaluates physical disorders, we are aware that patient's psychological burden should also be evaluated. We are now estimating the utility of this score chart for future standardization after necessary modification.

PM12.102
mir-221 as a pre- and post-operative plasma biomarker for laryngeal squamous cell carcinoma patients

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Larynx Cancer (LCa) comprises about 1% to 2.5% of all human neoplasms and it mostly originates from squamous cell carcinoma. Although distinct diagnostic and therapeutic approaches have been suggested for LCa, they are still controversial and more efforts are required for their effective use. MicroRNAs (miRNA) have been shown to play significant roles in the regulation of carcinogenesis and they were demonstrated to be present in a stable form in various human body fluids including plasma. Besides, miRNAs have been postulated in distinct studies as strong diagnostic or prognostic biomarkers.
In this study, we aimed to compare the miRNA profile of plasma samples obtained from 30 LCa patients (pre-operative and post-operative serum samples) and 30 healthy controls to identify a miRNA expression signature, which can be used to distinguish LCa patients from healthy individuals.
MiRNA profiling of eight plasma samples (four from pre-operative LCa samples, four from control individuals) were performed using miRNA microarray. Two of the significantly deregulated miRNAs were selected for further confirmation using quantitative reverse-transcription PCR (qRT-PCR). Statistical analysis was performed using Student's t-test.
Microarray profiling showed that miR-221 was upregulated and miR-133b was downregulated in LCa plasma samples. qRT-PCR analysis confirmed the overexpression of miR-221 in plasma samples of LCa. Further analysis qRT-PCR analysis demonstrated the reduced level of miR-221 to the normal level in the post-operative plasma samples.

PS12.103
Assessment of MLH1 promoter methylation in patients with endometrial cancer.

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Lynch syndrome (LS) is an autosomal dominant inherited cancer caused by a germline mutation in one of the DNA mismatch repair (MMR) genes: *MLH1*, *MSH2*, *MSH6*, or *PMS2*. Individuals with LS will commonly present with colorectal cancer (CRC), however female carriers also have an increased risk of developing endometrial cancer (EC). It is important to distinguish LS patients from sporadic cancer patients in order for appropriate disease surveillance and family members to be counselled.
The Molecular Oncology Laboratory currently performs a number of genetic tests aimed at identifying these patients, including immunohistochemistry (IHC), microsatellite instability (MSI) and MLH1 promoter methylation.
Methylation of specific regions of the promoter for the MLH1 gene results in epigenetic silencing of the gene and the patient may present with a similar clinical picture to a patient carrying a germ-line MMR gene mutation. Identification of MLH1 methylation will indicate that LS is unlikely and that MMR gene mutation screening is not required.
Methylation testing is carried out using a methylation-sensitive multiplex-ligation dependent probe amplification (MS-MLPA) technique which targets 5 sites in the MLH1 promoter corresponding to regions A, B, C and D and intron 1.
A review has been performed comparing the MLH1 methylation results at 5 sites in EC patients, with results in CRC patients. The incidence of EC patients with MLH1 methylation is 86%, with 97% of patients showing methylation across all 5 regions, compared with 74% of CRC patients. Therefore MLH1 promoter methylation testing in combination with IHC and MSI provides a useful diagnostic tool for identifying patients with LS.

PM12.104
Sanger sequencing of low amount of genomic DNA and FFPE DNA with PCR Primers derived from the Ion AmpliSeq cancer hotspot panel

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The introduction of Ion AmpliSeq™ panels for characterization of mutations occurring in tumor tissue has revolutionized translational oncology research. The Ion Ampliseq™ cancer hotspot panel version 2 (CHP v2) includes 207 actionable mutation targets in 50 genes and the Ion Oncomine™ cancer panel over 2000 mutations. A hallmark of these Ampliseq cancer panels is the low amount of input DNA needed which is critical when specimen material is limited such as with needle biopsy or FFPE samples. Typically, 10 ng of DNA is sufficient to produce informative sequencing data. Often, cancer-causing or promoting mutations are detected at low allele frequencies like 10-20 % compared to the normal allele. Many researchers wish to verify these low frequency mutations by an orthologous method such as Sanger sequencing on a capillary electrophoresis (CE) instrument. To that end, we have developed a workflow that enables the amplification and traditional Sanger sequencing of individual Ion AmpliSeq targets directly from the AmpliSeq library starting material. The method requires 1 µl (~ 5%) of the original AmpliSeq preamplification material. A dilution of this aliquot is used as template source for individualized PCR/sequencing reactions. We show that a selection of 48 targets from the CHPv2 panel could be successfully amplified and Sanger-sequenced from an Ampliseq library originally prepared from 10 ng of FFPE DNA. Furthermore, we show the successful Sanger-re-sequencing of all individual 24 targets covering the TP53 exons from the same sample processed and pre-amplified with the OncoMine AmpliSeq panel. This method enables researchers to reflex-test potential mutations from very material-limited specimen using Sanger CE sequencing.

PM12.106
NAB2-STAT6 fusion gene: Meningeal hemangiopericytomas and meningomas comparative study.

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Background: Meningeal hemangiopericytoma (MHPC) is a vascular tumor

arising from pericytes. Most intracranial MHPCs resemble meningiomas (MNGs) in their clinical presentation and histological features and may therefore be misdiagnosed, despite their important differences in prognosis.
Methods: We report 8 cases of MHPC and 5 cases of MNG collected from 2007 to 2011 from the neuro-Surgery and histopathology departments. All 13 samples were re reviewed by two independent pathologists and investigated by immunohistochemistry (IHC) using mesenchymal, epithelial and neuro-glial markers. Additionally, we screened all tumors for a large panel of chromosomal alterations using Multiplex Ligation Probe Amplification (MLPA). Presence of the NAB2-STAT6 fusion gene inferred by immunohistochemical staining for STAT6.
Result: Compared with MNG, MHPCs showed strong VIM (100% of cases), CD99 (62%), bcl-2 (87%), and p16 (75%) staining but only focal positivity with EMA (33%) and NSE (37%). The p21 antibody was positive in 62% of MHPC and less than 1% in all MNGs. MLPA data did not distinguish HPC from MNG, with PTEN loss and ERBB2 gain found in both. By contrast, STAT6 nuclear staining was observed in 3 MHPC cases and was absent from MNG.
Conclusion: MNG and MHPC comprise a spectrum of tumors that cannot be easily differentiated based on histopathology. The presence of STAT6 nuclear positivity may however be a useful diagnostic marker.

PS12.107
Genome-wide copy number analysis of pediatric malignancies.

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Neuroblastoma and hepatoblastoma are two cancers commonly characterized by chromosomal abnormalities such as copy number gains and losses, and regions of loss of heterozygosity (LOH). Typically, these abnormalities are detected through traditional cytogenetic and microsatellite assays. The heterogenous nature of these diseases suggests that additional genomic events, such as DNA variants and chromosomal gains/losses contribute to the disease etiology. With this in mind, we explored the use of the Affymetrix OncoScan FFPE Assay to improve on the diagnostic cytogenetics of these 2 cancer types. DNA was tested from FFPE pediatric tumor samples from 6 hepatoblastoma and 8 neuroblastoma cases. 6 of 8 neuroblastoma cases had MYCN amplification assessed by FISH. Cytogenetics was normal for the 6 hepatoblastoma cases, while Oncoscan testing revealed all 6 had copy number gains in 1q32 or 2p24, previously reported to be frequent in hepatoblastoma. For neuroblastomas, the cytogenetic and FISH findings were all confirmed by Oncoscan. In addition, multiple CNV and LOH were identified by Oncoscan. Furthermore, Oncoscan analysis of all tumor samples revealed 6 single nucleotide variants in 4 genes previously shown to be associated with neoplasia. The variants were independently validated using targeted panels on both the Ion Torrent and MiSeq DNA Sequencers. These results show that the OncoScan assay can accurately identify both chromosomal abnormalities and DNA variants that may otherwise be missed by traditional methodologies.

PM12.108
Homozygosity for MSH6 mutations Misdiagnosed as Neurofibromatosis Type 1

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Homozygosity for MSH6 mutations Misdiagnosed as Neurofibromatosis Type 1
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A 7 year old girl presented with multiple café au lait patches and mild developmental delay. She was the middle of three children of consanguineous Pakistani parents. She was given a diagnosis of Neurofibromatosis Type 1 (Nf1). At age 9yr, she developed seizures and a brain MRI showed a multi focal grade 3 anaplastic astrocytoma. She was seen in the genetics clinic. She had multiple café au lait patches and unusual speckled hyperpigmentation darker than usually seen in Nf1. She had very mild axillary and groin freckling. Her head circumference was on the 97th centile. Her grandmother was affected with bowel cancer. Homozygous pathogenic MSH6 mutations were found in our patient, confirming a diagnosis of mismatch repair cancer (Turcot) syndrome. No mutations were found in the Nf1 gene.
At age 11yr she developed two bowel malignancies. Her mother was also diagnosed with colon cancer.
There are only 6 reports of MSH6 homozygosity in the literature. Sadly the prognosis is poor. Problems include childhood intracranial astrocytomas or lymphomas, and colon cancer from 8yrs but usually in the teens or twenties. The parents and other family members will be MSH6 mutation carriers and require surveillance for bowel and endometrial cancers.
The presence of atypical signs, consanguinity or a family history of bowel cancer means that the diagnosis may not be Nf1 even in a child who meets the diagnostic criteria.

PS12.109
Genetic Testing Of Hereditary Breast And Ovarian Cancer Using Next Generation Sequencing And MLPA.

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Molecular diagnosis of Hereditary Breast and Ovarian Cancer (HBOC) is essential for the appropriate genetic counseling and to establish preventive screening strategies. *BRCA1* and *BRCA2* screening is offered to patients from high risk families. Several studies have demonstrated the potential of NGS in the field of research and genetic diagnosis.
Therefore, we designed a NGS targeted panel for genes associated with HBOC. Target regions (coding exons, splice sites and 5'-3'UTRs) for *BRCA1* and *BRCA2* were enriched and captured using SureSelect system (Agilent) and sequenced with MiSeq (Illumina). This panel was validated for diagnostic use with two HapMap cell lines and a set of cases with a previously known mutation detected by Sanger sequencing. After validation, samples from 180 patients with clinical suspicion of HBOC were tested in our laboratory. Clinically relevant findings were confirmed by Sanger sequencing. Negative results were tested for large genomic rearrangements using MLPA. A test report was issued in 5 weeks.
We identified 69 variants in 55 patients. All sequence variants could be confirmed by Sanger. Thirty-one were classified as disease-causing (8 novel mutations; 18 in *BRCA1* and 13 in *BRCA2*) and 38 as VUS. Thirty out of 180 patients (16.6%) were genetically diagnosed. The mutation spectrum included SNVs, small deletions/duplications, and large rearrangements.
Our results suggest that the combined NGS + MLPA panel may benefit appropriately selected patients for the molecular diagnosis of HBOC, as it has been proven as a fast, reliable and cost-effective alternative to Sanger sequencing.

PM12.110
Predisposition to breast cancer in NF1 occurs before, but not after, age 50y and is unrelated to NF1 gene mutation type or site

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Breast cancer (BC) is reported to occur in women with NF1 at increased incidence, especially those under 50y. We therefore ascertained women with NF1 and BC known to colleagues across Europe: total = 31. NF1 testing had found mutations in 26. Five had also undergone BRCA1/2 testing, revealing one BRCA2 mutation (BC age 46y; excluded).
Mean age of onset of BC was 44.7y (n = 20; range 30-65y; SD 10y). 15 cases occurred <50y, with 5y cohort standardised incidence ratios significantly raised in this group: 45-49y = 4.6(1.2-11.7), 40-44y = 5.8(1.2-17), 35-39y = 19.2(6.2-45), 30-34y = 27.0(5.4-79), but it is not possible to estimate the precise absolute risk from our data.
There was no difference in mutation type: truncating v. missense, compared to the NF1 LOVD (23, 3, 2158, 288; Fishers exact, ns).[www.lovd.nl/NF1] Mutations were distributed from exons 6 - 54, not significantly different from those on the LOVD, and there was also no relationship with either the GRD or ATP-binding domains.(Fisher's exact).

This is the first such study combining clinical and molecular data. We conclude that BC occurs in a subset of women with NF1 at a young age, and while the absolute risk is low, the relative risks appear high. The risk >50y is not increased. This predisposition does not appear to be a function of the underlying NF1 mutation type or site. This needs to be confirmed in other or larger cohorts, or possibly prospective studies, before recommendations might be made regarding surveillance. Genomic analysis may provide additional insights.

PS12.111

Next Generation Sequencing (NGS) analysis of 850 patients with Hereditary Breast and Ovarian Cancer: mutational spectrum in 94 cancer associated genes

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Breast and Ovarian Cancer (HBOC) is caused by germline mutations mainly in BRCA1 and BRCA2 (BC:5-7%, OC:11-15%), but mutations in low-penetrance susceptibility genes have been also demonstrated to account for significant cancer risk. Knowing the underlying molecular defects can be very valuable for diagnosis, guiding treatment and estimating recurrence risks. Here we report the results of panel-based NGS screening of 94 genes associated with hereditary cancer predisposition. 850 patients were consecutive tested for BRCA1/BRCA2 and a comprehensive diagnostic HBOC-panel including 38 genes. Patients with negative or inconclusive results were further analyzed for the remaining genes. Target enrichment with the Illumina TruSight Cancer Panel was followed by paired-end sequencing and bioinformatics data analysis. Diagnostic quality criteria ensured a minimal depth of 30X in at least 98% of the analyzed regions with 99.99% sensitivity and specificity in variant calling. For BRCA1/BRCA2 more stringent criteria were required (i.e. 100% coverage at >30X and deletion/duplication analysis). Results show that 8% of the patients have BRCA1/BRCA2 pathogenic mutations (class 4, 5), 7% were inconclusive (class 3) and 85% were negative (class 1, 2). Analysis of the HBOC-panel increased the diagnostic yield to 20%. Pathogenic variants were additionally found in ATM, BLM, BRIP1, BUB1B, CHEK2, FANCA, FANCC, FANCD2, NBN, PALB2, RAD51D, RECQL4 and TP53. 41% presented VUS and 39% were negative. Further screening of the remaining cancer predisposition genes revealed pathogenic variants in genes with no confirmed association with HBOC: BAP1, ERCC2, ERCC3 and MUTYH. Some of the genes were identified as causative only once, emphasizing the advantage of diagnostic panels for HBOC testing.

PM12.112

Multigene panel testing in hereditary breast and ovarian cancer patients

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Objective: Here, we summarize our experience over the last 2.5 years of HBOC-NGS-panel testing in a cohort of 364 patients that were referred to our institute for genetic testing.
Methods: DNA of all patients was enriched using a custom-design panel (Haloplex, Agilent) and sequenced on a MiSeq (Illumina). A median of 1.45 million reads was generated per sample resulting. Further analysis was limited to a set of 12 core genes. The median overall 20x-coverage was 98.01 %. Genes directly requested by the genetic counselor were complemented by MLPA as well as Sanger sequencing for low coverage regions (< 20x). The custom-design panel was continuously optimized and a reduction of missing bases by a factor of four was possible over time.
Results: Adding 10 more genes in diagnostic HBOC testing revealed the genetic underlying cause in additional 6% of all patients. In total, 19.4 % of our patients carried a mutation within one of the 12 core genes: 7.82 % in BRCA1, 4.85 % in BRCA2, 4.04 % in CHEK2, 1.35 % in ATM, 0.81 % in CDH1 and 0.27 % in NBN.
Conclusion: Taken together, we demonstrate that NGS is a fast and cost efficient genetic screening tool to analyze for variants in genes associated with the development of hereditary breast cancer However, with an increasing number of genes, correct variant interpretation is challenging and requires extensive genetic expertise. Furthermore, clinical guidelines for the new diagnostic BRCA genes still need to be agreed on.

PS12.113

Detection of NRAS and KRAS mutation in AML patients by Pyrosequencing

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Introduction: RAS genes play important roles in the regulatory processes of proliferation, differentiation, and apoptosis. RAS mutations result in occurrence of protein that induces uncontrolled cell proliferation and inhibit apoptosis. These mutations have been described in various human malignancies including acute myeloid leukemia (AML).
Materials and Methods: We demonstrated NRAS and KRAS mutation in AML patients by pyrosequencing. Twenty samples from AML patients were evaluated for mutation of these genes around the hot spots in codons 12, 13 and 61. Pyrosequencing was performed for detection of KRAS and NRAS mutation on the Pyromark Q24 system.
Results: Three of twenty patients (15%) had NRAS mutations and one of twenty patients (5%) had KRAS mutations. We found NRAS mutation in all of three codons; one case in codons 12, one case in codons 13 and one case in codons61. Both NRAS mutation in codons 12 and codons 13 were resulting in changes from glycine to asparagine (GGT>GAT) whereas NRAS mutation in codons 61 was resulting in changes from glutamine to histidine (CAA>CAT). Only one case that found KRAS mutation in codons 12 was resulting in changes from glycine to asparagine (GGT>GCT).
Conclusion: In this study, pyrosequencing is a powerful tool for detection of and KRAS mutation. The detection rate of NRAS mutation is higher than KRAS mutation in AML patients. However, a large number of AML cases is needed for further studying the molecular mechanisms in the pathogenesis and for prognostic value of the KRAS and NRAS mutations.

PM12.114

From oral cancer genetic and epigenetic alterations to clinical outcome prediction

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Introduction: Oral cancer is a clinical and molecular heterogeneous malignancy characterized by low survival and high mortality rates. The biological behavior of this neoplasm varies and tumors with the same clinic-pathological features often respond differently to the same treatment. Thus, the identification of genetic and epigenetic biomarkers is mandatory to improve diagnosis, prognosis, early detection of tumors and relapses and ultimately to delineate individualized therapy. Methods: Tumor tissues from 73 patients with oral tumors and gingival samples from 16 healthy donors were used as controls. Methylation-Specific Multiplex Ligation-dependent Probe Amplification (MS-MLPA) was conducted to screen copy number alterations and DNA methylation patterns in 54 tumor suppressor genes. Results: From the 54 tumor suppressor genes analyzed those that most frequently exhibited hypermethylation were WT1, MSH6, PAX5 and GATA5. The most frequent copy number alterations were located at chromosomes 3, 9, 11, 12, 16, 17 and 19, highlighting, for example, CTNNB1 and FHIT genes with losses and CDH1, BRCA1, PYCARD, STK11 and CHFR genes with gains. It was possible to find some statistically significant associations among these alterations and the clinic-pathological features of the patients, such as metastases and also smoking habits. Conclusion: The combination of genetic and epigenetic studies together with the pathological diagnosis seems to be mandatory not only to early detect these tumors and relapses but also to predict their clinical behavior. In this way, the increasing knowledge about the genetic and epigenetic mechanisms associated to oral cancer, opens new possibilities at the diagnostic, prognostic and treatment level.

PS12.115

Impact of multi-gene testing in ovarian carcinoma patients selected for personal and/or family history

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Background: 20-25% of Ovarian Carcinomas (OC) are the result of an inhe-

rited predisposition. Besides *BRCA* and MMR genes, several additional contributing genes have been identified. Multi-gene panel testing provides an efficient method for a comprehensive assessment of genes associated with cancer risks. We report our experience of effective multi-gene testing in OC patients selected during genetic counselling (GC) for personal and/or family history.
Methods: The myRisk panel from Myriad Genetics Laboratories (25 clinically significant genes) was run in consecutive patients who met one of the following criteria: diagnosis before age 40, multiple primary tumors and/or at least one first/second degree family member affected by a distinctive cancer of known hereditary cancer syndromes at any age.
Results: Twelve out of 20 OC patients who underwent GC from September 30, 2014 to December 31, 2014, met inclusion criteria: 5 of 12 (42%) carry deleterious mutations (2 in *BRCA1*; 1 each in *BRCA2*, *MSH6*, *PMS2*). Ten variants of unknown significance (VUS) (2 in *NBN*; 1 each in *PMS2*, *BRCA2*, *ATM*, *APC*, *PTEN*, *MLH1*, *P53*, *BRIP1*) were identified in eight subjects (8/12; 67%): of these, three also carried a deleterious mutation and one was a suspected somatic mosaicism. Moreover, two deleterious monoallelic *MUTYH* carriers were detected.
Conclusion: The use of multi-gene panels including *BRCA* and MMR genes allows the "one-step" detection of actionable mutations in a large fraction of OC patients with second primaries and/or family history. The identification of VUS is stimulating from a scientific perspective but should be appropriately managed during GC.

PM12.116
High uptake of risk-reducing bilateral salpingo-oophorectomy in BRCA1 and BRCA2 gene mutation carriers at The Royal Marsden

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Background: Risk-reducing bilateral salpingo-oophorectomy (RRBSO) in BRCA1 or BRCA2 mutation carriers is highly effective in reducing ovarian cancer (OC) risk, by up to 95%. However, reported rates of RRBSO uptake vary widely. We recommend consideration of RRBSO to BRCA1 carriers aged ≥40yrs and BRCA2 carriers aged ≥45years. Here we have assessed RRBSO uptake amongst BRCA1 and BRCA2 carriers at Royal Marsden (RM) hospital.
Methods: We reviewed the electronic patient record for carriers in the RM Genetics unit BRCA carrier register. Our primary endpoint was uptake of RRBSO. Secondary endpoints were rates of occult malignancy at RRBSO and reasons for not undergoing RRBSO.
Results: 858 female mutation carriers were identified, of whom 557 met our age criteria (304 BRCA1, 253 BRCA2). 73 women had been diagnosed with OC, leaving 265 BRCA1 and 219 BRCA2 carriers. Of these, 234(88%) BRCA1 carriers and 192(87%) BRCA2 carriers underwent RRBSO. Occult OC was identified in three BRCA1 carriers; two further BRCA1 carriers had intraepithelial neoplasia. Reported reasons for not undergoing RRBSO were personal choice (36 women), metastatic breast cancer (12 women) and preservation of fertility (six women - all BRCA1 mutation carriers in early 40s). We do not undertake NHS ovarian surveillance because of unproven efficacy but of those who declined RRBSO, six have private ovarian surveillance.
Conclusions: We observed a high rate (87%) of RRBSO uptake amongst BRCA1 and BRCA2 carriers at the Royal Marsden.
This work was funded by the RM/ICR NIHR BRC.

PS12.117
Analysis of PALB2 and other predisposing genes in patients without mutations in the BRCA1 and BRCA2 - benefits for counseling in families with recurrent breast cancer

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Mutations in BRCA1 and BRCA2, main predisposing to breast (BC) and ovarian cancer (OC) genes, were found in less than half of the families suspect for hereditary form of BOC; mutation carriers in either gene constitute only about 25% of the population of high risk patients. Other genes were identified (ATM, CHEK2, NBN, PALB2 etc.), whose mutations can increase the risk of BC in BRCA1 and 2 negative patients. These were described as medium-risk genes or genes with moderate penetrance. Mutations in the ATM and NBN genes were recorded with low frequency in Czech population, and therefore they can not be recommended for routine clinical testing. In contrast, the analysis of c.1100delC mutation and large exon 9 and 10 deletions in CHEK2 gene were recommended for practice in the CzR; currently, missense variants of this gene are subject of focused research. PALB2 gene testing in patients with BOC, but with a normal sequence in BRCA1 and 2 showed that point mutations and major reconstruction of PALB2 (16 in total, 3.9%) can cause the disease in Czech patients (n = 409). Most of these mutations were observed in the subgroup of patients with the inherited form of BC - here occurrence reaches 5.5% (13/235). Thus, the significance of PALB2 gene mutation analysis in this particular subpopulation is comparable to that of the BRCA2 gene. We believe that the PALB2 should be included in the algorithm of clinical genetic testing in BC patients negative for mutations in the BRCA1 and 2.
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PM12.118
Germline and somatic HIF2A mutations associated with Phaeochromocytoma and Paragangliomas.

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Phaeochromocytomas (PCCs) and paragangliomas (PGLs) are rare autonomic nervous system tumours, which typically hypersecrete catecholamines. A notable feature of PCCs/PGLs is the high incidence of inherited cases, such that germline mutations in at least 12 susceptibility genes can be detected in 30-40% of all cases. Recent gene expression profiling studies have shown that a large proportion of inherited cases show activation of the hypoxic gene response pathways. It has also been shown that loss of function mutations in VHL and SDH subunit genes lead to the stabilisation of the hypoxia-inducible factor (HIF) proteins. These HIF proteins are a family of transcription factors (HIF-1, HIF-2 & HIF-3) that bind to and activate multiple genes associated with angiogenesis, glycolysis and cell growth. The stability of the HIF proteins is dependent on two specific proline residues located in the oxygen dependent degradation domain, that are hydroxylated by the oxygen dependent prolyl hydroxylases (PHDs). Once hydroxylated, the HIF proteins can be targeted for proteasomal degradation by the VHL complex. Recently, several groups have reported gain of function mutations in HIF2A/EPAS1 in PCC/PGL cases, which result in the stabilisation of the HIF-2 protein. To date, the majority of the HIF2A mutations have been detected as somatic mutations, with little evidence of germline mutations. Here we report the finding of six novel variants, four of which were found present in the germline, and cluster around the secondary hydroxylation site. We also present functional data, which provides further evidence that these variants are pathogenic.

PS12.119
Whole genome expression, gene network and pathway determination by microarray analysis in pituitary tumors

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Introduction: Pituitary tumorigenesis suggests that genetic alterations that associated with cell cycling, cell proliferation and angiogenesis may play major role in the initiation and promotion of pituitary adenomas.The aim of this study consists in identification of novel genes and/or pathways with potential roles in pituitary tumorigenesis.
Materials and Methods:RNA samples were obtained from a hundred pituitary tumors and three pituitary tissues taken from healthy cadavres.RNA quality, based on the 28S,18S ribosomal RNA ratio, was assessed with an Agilent Bioanalyzer (Agilent Technologies,Palo Alto,CA,USA) and RIN scores arrounds 4 were included.Tumor size and invasiveness were defined on the basis of preoperative magnetic resonance imaging (MRI) and surgical findings.On these basis; 24 microadenoma,30 microadenoma and 46 invasive pituitary adenoma were analyzed.RNA samples were hybridized with microarray chips (Agilent Human 4X44K Oligo Microarrays).Gene expressions, canonical pathways and network analyisis were performed using GeneSpring GX13.0 and Ingenuity Pathway Analysis software.
Results: Microarray analysis identifed 40 downregulated and 58 upregulated genes in invasive adenoma; 40 downregulated and 51 upregulated genes in macroadenomas; 41 downregulated and 42 upregulated genes in microadenomas.The canonical pathways significantly regulated were cel-

lular movement,cellular growth and proliferation,cell cycle,cell death and survival,cancer, nervous system development and function.
Conclusion: Some gene profiles showed good agreement with our data such as IDH1 and CLU.In addition, GATA3, RACGAP1, SSX2IP and PTPRA genes were overexpressed in our invasive and macroadenomas.It's suggested that these genes might be used as a molecular marker set for invasive,proliferation and agreessiveness in pituitary tumors.These candidate genes will be validate with RT-qPCR method in our future study

PM12.120

Frequent coexistence of hyperdiploidy and high-risk cytogenetic changes in plasma cell myeloma in Chinese

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Introduction
We report our experience in the application of fluorescence in-situ hybridization (FISH) to characterize genetic abnormalities in plasma cell myeloma (PCM) among Chinese patients.
Materials and Methods
A total of 100 patients with PCM were studied. Their age ranged from 28 to 87 years (median and mean = 59) with a male to female ratio of 1.45:1. FISH was performed on CD138-sorted bone marrow samples.
Results
High-risk genetic changes were found in 46 patients (Table 1). Some patients had more than one unfavourable factor. 15 patients had concomitant high-risk genetics and hyperdiploidy while 31 patients had high risk genetics without hyperdiploidy. 22 patients had died of their diseases. 59% (13/22) were in the high-risk group with only two having concomitant hyperdiploidy, suggesting that hyperdiploidy might have ameliorating effect. Furthermore, 14 patients were relatively young in age (60 years or below) with five having high-risk genetics but without hyperdiploidy.
Conclusions
Our results suggested a beneficial effect of concomitant hyperdiploidy in plasma cell myeloma with high-risk genetics. This will help identify patients for targeted personalized therapy.
Table 1. Incidence of high risk genetics and hyperdiploidy.

	del(1)p	1q gain	t(4;14)	t(14;16)	del(17)p	hyperdiploidy	Combined high risk genetics and hyperdiploidy
% cases	7.8	40.6	16.0	5.1	12.1	32.3	16.1

PS12.121

Assessment of JAK2V617F Mutation in CD133/ CD34 Cell Compartments

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Introduction; BCR-ABL negative Myeloproliferative Neoplasms (MPNs) are clonal stem cell disorders which are the results of genetically transformed stem cells and risks of increased *leukemic* transformation. MPN includes polycythemia vera (PV), essential thrombocythosis, primary myelofibrosis. JAK2V617F mutation occurs in MPN patients with a high range between %50-98. The aim of study is to analyse and compare mononuclear and specific stem cell compartment of PV patients having JAK2V617F mutation.
Materials and Methods; The mononuclear cells were isolated by ficoll–gradient method in three PV patients' peripheral blood samples taken by phlebotomy. Those cells were stained by a group of cell surface marker and selected in cell sorter. Initially $CD45^-$ cells were gated and then $CD133^+CD34^-$, $CD133^-CD34^+$, $CD133^+CD34^+$, $CD133^-CD34^-$ stem cell compartments sorted by cell sorter.
In order to investigate JAK2V617F mutation in mononuclear cells and $CD133^+CD34^-$, $CD133^-CD34^+$, $CD133^+CD34^+$, $CD133^-CD34^-$ stem cell compartments, allel specific Nested PCR was performed.
Results; JAK2V617F mutation analysis were compared between mononuclear cells and different stem cell compartments. According to this comparison there has been no difference for JAK2 mutation screening between mononuclear cells and different stem cell compartments.
Conclusions; We aimed to investigate the presence of the JAK2V617F mutation in both stem cells compartments and mononuclear cells of PV patients in this study.
As a result of the detailed research in terms of JAK2 mutation is revealed that there has been no differences between stem cells compartments and mononuclear cells of PV patients.
This research performed limitied number of patient the increased number of patient would enhance our knowledge.
Our project supported by TUBITAK 1001 grant.Project No:112S483

PM12.122

Therapy response monitoring in patient with prostate cancer using plasma-Seq approach

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Background: Prostate cancer is the most common malignancy in males. Prostate cancer progression can be inhibited by androgen-deprivation therapy but nearly all patients progress to castration-resistance prostate cancer (CRPC). There still remains the unsolved question how patients can be best matched with targeted therapies according to characteristics of their tumor genome.
Methods: We analyzed a total of 73 plasma samples from 20 CRPC and 9 CSPC (castration-sensitive prostate cancer) patients using our previously established plasma-Seq approach including low coverage whole-genome sequencing to establish copy number profiles and targeted resequencing of prostate cancer associated genes on an Illumina MiSeq platform.
Results: Analysis of plasma DNA from prostate cancer patients revealed a variety of copy number changes characteristic for prostate cancer. In a subset of patients we were able to observe the emergence of AR gene amplification at the time of progression from CSPC to CRPC. AR gene amplifications were found in approximately 70% of CRPC patients. Furthermore, we were able to monitor the evolution of novel focal amplifications and clonal shifts due to therapy changes in one third of the patients. Moreover, plasma DNA analyses reflected the treatment response to second line treatment, i.e. cytotoxic chemotherapy.
Conclusions: Our study showed that analyzing plasma DNA offers a non-invasive method to monitor patients' therapy response and may identify the occurrence of novel changes associated with resistance against a given therapy. Plasma DNA analyses may evolve to a novel tool for monitoring of patients with cancer and for development of personalized medicine.

PS12.123

Testicular lipomatosis as a presentation of PTEN Hamartomatous Tumour Syndrome - A case report

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PTEN hamartoma tumour syndrome (PHTS) is associated with a variety of clinical manifestations including macrocephaly, characteristic skin lesions and other benign or malignant tumours in various tissues such as the thyroid gland, breast, endometrium, bowel and fat. Testicular lipomatosis has been reported in a series of individuals with PTEN mutations but it is not known what proportion of patients presenting with this condition have PTEN mutations. Here we present a case of unilateral testicular lipomatosis in a 23 year old male, referred to clinical genetics services due to the fact that macrocephaly was noted in conjunction. He had previously had two lipomas removed from the neck area at age 5 and examination revealed a lesion consistent with a lipoma on his right foot. No other features of PHTS were noted on examination or in the family history. Germline genetic analysis revealed a heterozygous G to C base substitution within the invariant splice acceptor site for exon 8 that is considered to be pathogenic. We suggest that testicular lipomatosis in conjunction with a characteristic feature of PHTS should prompt consideration of PTEN analysis.

PM12.124

The first consensus based guideline on PTEN Hamartoma Tumor Syndrome (PHTS) in the Netherlands

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Here, we present the new Dutch consensus-based guideline on PTEN Hamartoma Tumor Syndrome (PHTS). The guideline is designed to improve early diagnosis, surveillance and care of patients with PHTS.
Development of the guideline has been a collaborative effort of a multidis-

ciplinary group of health care professionals and patient representatives. Several questions on detection, surveillance and care were defined and answered by pubmed literature searches.
The guideline describes clinical criteria for PHTS diagnosis and criteria for referral for genetic counselling and/or DNA analysis of the PTEN-gene.
The following advices for surveillance are given: follow up of development is recommended from diagnosis until 18 years, including control of the thyroid. Adult male and female patients are advised to have surveillance of the thyroid above the age of 18 and of the colon above the age of 40. For female patients, surveillance of the breasts is recommended from age 25 and endometrial screening from age 30.
It is recommended to inform the patients about the benign features of PHTS and the increased risk of cancer, for which information on the exact risks is not reliable. This is because the risks in the cohort studies are probably an overestimation due to bias. Patient information can also be given on paper, websites or social media.
Surveillance is preferred in a specialized university medical centre. To support the surveillance a roadmap has been developed.
This guideline on PHTS describes recommendations on detection, surveillance, and care of patients with PHTS and aims to improve quality of care.

PS12.125
RAD51 paralogs in breast and ovarian cancer predisposition

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RAD51 paralogs (*RAD51B*, *RAD51C*, *RAD51D*, *XRCC2*, *XRCC3*) have recently been implicated in breast and ovarian cancer predisposition: *RAD51C* and *RAD51D* in ovarian cancer, *RAD51B* and *XRCC2* in breast cancer through monoallelic germline deleterious mutations. Previous studies were mostly conducted on family cases selected for high predisposition risk. In addition, *XRCC3* polymorphisms have been associated with breast cancer in case-control studies but no deleterious mutation has been reported.
To better estimate the contribution of *RAD51* paralogs in breast and ovarian cancer predisposition, these genes were analysed in 2,991 consecutive unrelated patients diagnosed with breast and/or ovarian cancer and previously tested negative for *BRCA1/2* mutations. Deleterious truncating mutations were detected in the five *RAD51* paralogs: *RAD51B* (n=4), *RAD51C* (n=14), *RAD51D* (n=7), *XRCC2* (n=2), and *XRCC3* (n=5). The mutation rate was 1.1% (32/2,991 patients). Likely deleterious missense variants were also detected in the five *RAD51* paralogs at a lower rate: 0.5% (15/2,991 patients).
This is the first study of the five *RAD51* paralogs conducted on a large series of consecutive unrelated patients, allowing a better evaluation of mutation rate (overall rate: [1.1-1.6%]); not surprisingly, *RAD51* paralog mutation rate is lower in this series of unselected patients than previous estimates in patients at high predisposition risk. This is also the first report of *XRCC3* truncating mutations in breast and ovarian cancer predisposition. To assess more accurately the *RAD51* paralog mutation rate, functional assays for interpretation of missense variants are ongoing. This study constitutes a sound basis for penetrance risk estimates through the genetic testing of relatives of mutation carriers.

PM12.126
Breast cancer molecular classification based on DNA methylation assessed by reduced representation bisulfite sequencing

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We have developed a modification of the RRBS method applicable to assess methylation in large collections of DNA samples within biomedical research. Briefly, our modification further reduces the size of the representation to be sequenced simultaneously increasing the inner fraction of CpG islands and shores. By use of this approach we have obtained RRBS data for 80 breast cancer (BC) samples, 6 BC cell lines and 10 samples of normal breast tissue. To date, this is the most comprehensive collection of RRBS data for a set of tumors of the same organ. Cluster analysis of this data distinguishes at least five molecular subtypes of breast tissues (cells) based on the CpG methylation. Specific subtypes are assigned to normal breast tissues, BC cell lines, triple negative and HER positive (indistinguishable based on the present dataset), and two independent groups of luminal BC regardless of their HER status. The most pronounced differences of the DNA methylation patterns are observed between normal breast tissues and BC cell lines, the latter presenting the highest CpG methylation levels within the samples, notwithstanding different immunoprofiles (LumA, LumB and triple negative).
One of the most striking findings is separation of the luminal tumors into two clusters differing by the density of CpG methylation, independently on HER status (LumA or LumB). This separation may reflect differences in the tumor biology between groups and requires further research in terms of utility for prognostication and treatment that may arise from the DNA methylation markers.
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PS12.127
Resveratrol promotes apoptosis, autophagy and suppressed cell division via upregulated autophagy-related gene, caspase 3 and Cyclin dependent kinase inhibitor in human K562 cells.

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Chronic myeloid leukemia (CML) is a malignant disorder of the haematopoietic stem cell arisen from the reciprocal translocation between the breakpoint cluster region (BCR) gene on chromosome 22, and the Abelson (ABL) murine leukemia virus gene on chromosome 9, t(9;22)(q34;q11), resulting in the formation of Philadelphia chromosome.
Resveratrol is a natural phytoalexin and induces apoptosis, erythroid differentiation and autophagy in leukemic cells.
In this study we aimed to evaluate the cytotoxic, apoptotic and autophagic effect of resveratrol in CML cells by questioning gene expressions which are associated with CML progression.
K562 cells were treated with resveratrol time and dose dependent manner and cytotoxicity was evaluated by using WST-1 assay. The RT-qPCR is used for gene expression analysis. Gene expression levels were evaluated by using RT2 Profiler PCR Array.
Significant increase was observed in K562 cells treated with resveratrol according to control. The gene expression levels of ATG5 (autophagy-related gene), CASP3 (caspase 3) and p27KIP1(Cyclin dependent kinase inhibitor) were increased respectively 10.41, 8.63 and 36 fold via resveratrol.
Our findings showed that Resveratrol induced apoptosis and autophagy in K562 cells. Resveratrol upregulated caspase-3 activation in apoptosis and modulation of Atgs in autophagic pathway in K562 cells. Also, resveratrol regulates autophagosome formation via upregulated-ATG5 expression. Cdk inhibitor p27KIP1 is a tumor suppressor gene and includes in cell cycle process. Thus upregulated p27KIP1 expression in K562 cells induced prevented progression from G1 to S-phase and suppressed cell division. Therefore, Resveratrol prevented the formation of tumors in K562 cells. These results provide that Resveratrol may be a therapeutic candidate for chronic myeloid leukemia treatment.

PM12.128
Identification of novel mutations in RB1 gene in retinoblastoma patients

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Purpose: Retinoblastoma initiated by biallelic loss of RB1 can be successfully treated. However, multimodal treatment failures results in enucleation. We hypothesized that mutations may contribute to treatment failure.
Methods: We studied 2 retinoblastoma cases with multimodal treatment failure. Clinical findings, pathologic features following enucleation and treatment were documented. DNA extracted from patient tumor, adjacent normal and blood was subjected to direct sequencing for mutation detection in RB1 gene. Sequences were analysed using DNAstar software. Effect of variants was predicted using Polyphen-2 and mutation taster software.**Results:** Patient 1, 17 month male presented with unilateral sporadic group D. After 6 cycles of chemo and radiotherapy, patient developed recurrent active tumor. Pathology showed vitreous seeding, viable intraocular tumor without high risk features. Patient 2, 13 month male with sporadic bilateral group D retinoblastoma, right eye (enucleated). Left eye received 9 cycles of chemo and radiotherapy but progressed to stage E, also enucleated. Pathology showed viable intraocular tumor, focal choroidal invasion and optic nerve invasion with negative surgical margin. We are reporting two novel mutations in the RB1 gene. Patient 1, had insertion of two base pairs that resulted in frameshift and protein truncation (p: E97Rfs*15). Patient 2 showed a stop mutation

early in the protein sequence (p: Q35*).
Conclusion: These novel germline truncating mutations in patients with advanced retinoblastoma resulted in poor ocular survival despite extensive treatment. Truncating mutations represent over 50% of all *RB1*mutations. It is possible this type of mutations responsible for tumor resistance. Screening additional patients with similar clinical history is required to confirm this data.

PS12.129
From guideline recommendations to familial cancer risk assessment decision support in primary care: US Experience

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Introduction: There are several U.S. guidelines for identifying and managing risk for hereditary cancer syndromes. While fundamentally similar, they each differ in details that can lead to differences in assignment of risk levels (Orlando, AJMG, 2014). In order to better understand how these differences in guidelines affect individuals, we describe preliminary results from a nationally representative population of individuals.
Materials and Methods: Eligible participants are patients visiting enrolled primary care clinics at: Duke University, Medical College of Wisconsin, Essential Rural Healthcare Institute, David Grant Medical Center, or University of North Texas. Enrolled participants enter their personal and family health history information into MeTree, a patient facing risk assessment and clinical decision support web-service.
Results: To date 222 participants have completed MeTree. Characteristics include 62% female; 89% white, and mean age 59.6 (range 24-84). The mean no. of relatives entered is 13.3 (range 6-62). In an initial assessment of one guideline 1.8% (n=4) had > 20% lifetime risk of breast cancer and 33.3% (n=148) met criteria for a possible hereditary cancer syndrome. Assessment of risk level using three additional hereditary cancer syndrome guidelines will be performed on the enrolled population as of May 30th, 2015 and data presented showing discrepancies in those identified as at risk by each guideline.
Conclusion: Hereditary cancer risk assessment guidelines are developed based on expert opinion and experience, which has the potential to lead to variations in recommendations by different agencies. Establishing a database of family health histories linked to outcomes can inform guideline development and lead to greater consistency and accuracy in risk assessment algorithms

PM12.130
An assessment of known skin cancer risk variants in a population of renal transplant patients

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The risk of a kidney transplant recipient developing skin cancer is approximately 33 times higher than the general public. Multiple robust genetic loci for skin cancer have been identified via large genome-wide association (GWA) studies. We wished to test the hypothesis that these loci are risk factors for skin cancer in post-transplant populations. We were also interested in determining if the odds ratio changed in the transplant population, compared to the general population in which they were discovered. We identified 21 robustly associated SNPs from the GWA studies and tested these SNPs in a cohort of 325 cadaveric kidney transplant recipient patients. The cohort was cross referenced with the Irish cancer registry resulting in the identification of 94 cases that developed skin cancer post-transplantation and 231 controls. Illumina 660K bead-chip data was available for all 325 individuals. Logistic regression and survival analysis was applied to test for correlation with skin cancer.
A nominally significant association was found with rs885479, a SNP found in the *MC1R* gene (p= 0.0157) in the survival analysis. The variant was found to have the same direction of affect as described in the original study and the odds ratio was higher. The presence of one or more copies of the minor allele caused a significant decrease in time to develop skin cancer post renal transplant (hazard ratio = 2.06). However, none of the variants survived Bonferroni correction for multiple testing. We are now seeking to extend this analysis to additional patient cohorts.
This work is funded by the Irish Research Council for Science, Engineering and Technology.

PS12.131
Development and validation of the Oncomine Cancer Research Panel (OCP), a scalable next-generation sequencing system for assessing recurrent somatic alterations in solid tumors

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Treating cancer effectively requires an understanding of the molecular alterations driving each patient's tumor. Targeted sequencing efforts that characterize prevalent somatic alterations and require limited sample input may provide an effective diagnostic approach. Herein, we describe the design and characterization of the Oncomine Cancer Research Panel (OCP) that includes recurrent somatic alterations in solid tumors derived from the Oncomine™ cancer database. Using Ion AmpliSeq™, we designed a DNA panel that includes assays for 73 oncogenes with 1,826 recurrent hotspot mutations, 26 tumor suppressor genes enriched for deleterious mutations, as well as 75 genes subject to recurrent focal copy gain or loss. A complementary RNA panel includes 183 assays for relevant gene fusions involving 22 fusion driver genes. Recommended sample inputs were 10 ng of nucleic acid per pool. Sequencing libraries were analyzed on an Ion Torrent Personal Genome Machine. Initial testing revealed an average read depth of > 1,500X with > 95% uniformity and on target frequency. The panel was shown to reliably detect known hotspots, insertions/deletions, gene copy changes, and gene fusions in molecular standards, cell lines and formalin-fixed paraffin embedded clinical specimens. Retrospective analysis of large clinical cohorts has been completed and the results of analysis of 100 lung cancer and 100 prostate cancer cases will be summarized. In addition, a prospective cohort of 100 clinical samples from the University of Michigan Molecular Diagnostics laboratory was profiled with OCP. Overall, we achieved >95% sensitivity and specificity for detection of KRAS, EGFR and BRAF mutations and ALK gene fusions.

PS12.135
Association between GWAS-derived rs966423 genetic variant and overall mortality in patients with differentiated thyroid cancer

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Background: Five germline genetic variants (rs116909374, rs965513, rs944289, rs966423, rs2439302) have been associated in genome-wide association study (GWAS) with increased risk of thyroid cancer, but their role in mortality of patients with thyroid cancer has not been established.
Study design: Retrospective study of 1836 patients with differentiated thyroid cancer (1643 women and 193 men) with a median age at diagnosis of 49 years (interquartile range, 38-57years) and an overall median follow-up time of 8.7 years (interquartile range, 5-12.5years) after initial treatment at a single comprehensive cancer center between 1990-2013.
Results: Among 5 variants, rs966423 was associated with increased mortality, which was 6.4% (33/518) vs. 3.7% (47/1259) in TT-carriers vs. CC/CT carriers (P=0.017). Deaths per 1000 person-years were 6.81 vs. 4.01 in TT vs. CC/CT patients (HR=1.6; P=0.038) after adjustment for age at diagnosis, and sex. Importantly, the association of rs966423 with mortality remained significant when lymph-node metastasis, extrathyroidal invasion, angioinvasion and distant metastasis were included in the model (HR=1.89; P=0.014). A higher rs966423-associated patient mortality was also observed in several clinicopathological subcategories, e.g. in TT patients with angioinvasion (HR=3.48; P<0.001).
Conclusions: rs966423-TT genotype was significantly associated with increased overall mortality among patients with thyroid cancers. Contrary to *BRAF* mutation and other somatic changes putatively associated with patients mortality, the status of germline rs966423 is known before the treatment, and might be used in management of mortality risk by means of modification of therapy, nevertheless it requires further investigation in large prospective studies before it is ready for clinical application.
Support: Polish National Science Centre (DEC-2013/11/B/NZ3/00193); European Commission (FP7-REGPOT-2012-CT2012-316254-BASTION).

PM12.136
An investigation of mitochondrial DNA haplogroups in patients with thyroid cancer

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Introduction: Recent studies have shown that mitochondrial DNA (mtDNA) haplogroups could be associated with cancer development. The present study aimed to analyze mtDNA control region variation of 114 patients with thyroid follicular adenoma (TFA), 121 patients with papillary thyroid cancer (PTC) and 212 healthy controls in order to investigate whether mtDNA haplogroups contribute to the onset of cancer.
Materials and Methods: The two hypervariable segments HVSI and HVSII of the control region were sequenced on ABI3130xl platform for all samples. The unambiguously classified samples were assessed by means of PCR-RFLP using a hierarchical system. We performed statistical analysis including Pearson's chi-square test, Fisher's exact test and binary logistic adjusted by age and gender.
Results: The haplogroup K appeared underrepresented in TFA and PTC patients when compared to healthy controls (p = 0.010; OR = 0.115; 95% CI = 0.015 – 0.883 and p = 0.010; OR = 0.109; 95% CI = 0.014 – 0.839, respectively). The haplogroup HV was associated significantly with increased TFA risk (p = 0.032 OR = 2.697; 95% CI = 1.052 – 6.910).
Conclusions: We found that HV haplogroup may be associated with increased risk of developing TFA, and K haplogroup can have a protective effect for TFA and PTC.
The study was supported by CNCS-UEFISCDI grants PN-II-ID-PCCE 2011-2-0013 and PN-II-PT-PCCA-2011-3.2-1337.

PS12.137
A polymorphism in miR-146a tailors genetic predisposition to differentiated thyroid cancer, modulates its clinical outcome and alters proliferation of tumor cells.

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Introduction: Rs2910164 in mir-146a was among the first polymorphisms described in a microRNA gene. The G-C substitution leads to reduction of the amount of miR-146a and to regulation of different set of target genes. The polymorphism was further shown to predispose to papillary thyroid carcinoma (OR=1.62). However, no study analyzed the influence of the SNP on the clinical outcome of thyroid cancer patients.
Materials and Methods: The rs2910164 was genotyped using the Sequenom technology in blood-derived DNA from 2872 patients treated for differentiated thyroid cancer (overall median follow-up time 8.7 years). Genotyping in thyroid tissue samples was performed using the Taqman assay. The influence of miR-146a on the thyroid cancer cell lines was analyzed in K1 cells with induced overexpression or silencing of pre-miR-146a.
Results: The germinal C allele in rs2910164 was associated with higher mortality among patients with follicular variant of papillary thyroid carcinoma. Deaths per 1000 person-years were 25.64 vs. 4.72 in CC vs. GG/CG patients (HR=5.88; *P*=0.008). Moreover, a somatic G-C mutation in mir-146a was observed in 6.5% of the analyzed tumor samples. *In vitro* studies showed that miR-146a significantly increased proliferation of thyroid cancer-derived cell line and the presence of the variant C allele resulted in lower proliferation rates, possibly switching the cells towards migration.
Conclusions: Rs2910164 in miR-146a predisposes to thyroid carcinoma, causes higher mortality of thyroid cancer patients and undergoes somatic mutations in tumor. G-C transition might be responsible for increased metastatic potential and aggressiveness of cancer.
Support: Polish National Science Centre (DEC-2013/11/B/NZ3/00193); European Commission (FP7-REGPOT-2012-CT2012-316254-BASTION).

PM12.138
The TSH-beta gene confers a risk for differentiated thyroid cancer in the Saudi population

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Background: The thyroid stimulating hormone (TSH-beta) is a heterodimeric glycoprotein which regulates the secretion of the thyroid hormone and therefore thyroid function. In the present study, we evaluated the role of the TSH-beta polymorphisms as risk factors for development of differentiated thyroid cancer (DTC).
Methods: We first sequenced the TSH-beta gene, performed a preliminary association study involving 96 cases versus 96 controls, and then selected four variants for the association studies in a larger population size using Taqman assays.
Results: In the preliminary association study, three of the discovered SNPs, rs72695872_C>T [Odds ratio (95% Confidence Interval)=1.78(1.07-2.97); p=0.029] and rs1321108_A>G [2.15(1.28-3.62); p=0.004] in the promoter region as well as the intronic rs17477369_T>A [2.50(1.03-6.06); p=0.048] were associated with DTC in a causative fashion. These results were confirmed in a larger study involving 507 cases versus 597 controls, at similar or higher significance levels. Also, 4-mer haplotype AGGT (x2=7.36; p=0.0067) and its 3-mer flanking derivatives GGT (x2=11.71; p=0.0054) and AGG (x2=7.45; p=0.0063) conferred significant risk for acquiring thyroid cancer. Another 4-mer haplotype, GAGT (x2=11.21, p=0.0008) and its 3-mer derivative AGT (x2=11.71; p=0.0006) displayed equipotently protective properties against the disease, pointing to changes in the rs1321109_G>A, rs10776792_A>G and rs1321108_A>G as the probable determinants of these observations.
Conclusion: Our results indicate that the TSHβ constitutes a potential risk for DTC in the Saudi population.

PS12.139
Identification of novel germline mutations in the TP53 gene in Swedish families

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Introduction: A constitutionally inherited mutation in the *TP53* gene increases the risk of cancer. It causes an autosomal dominant inherited cancer syndrome named Li-Fraumeni syndrome (LFS). LFS is associated with a risk of a wide range of tumors, however, mainly including breast cancer, brain tumors, adrenocortical carcinomas, as well as osteo- and soft tissue sarcomas. In some families pediatric tumors are more frequent, while in others only breast cancer in adult is seen. The underlying cause for this variation remains unknown.
Results: In an attempt to understand the cancer for this genotype-phenotype correlation and to design adequate surveillance program for these families, we formed The Swedish Clinical *TP53* Study Group. The Swedish constitutional *TP53* cohort consists up to date of 32 families: Two-third of the families were diagnosed as either LFS or LFS-like and the remaining families with exclusively hereditary breast cancer. We found five novel germline mutations i.e. previously not reported. Four of these were frameshift mutations that disrupted the major transcript of *TP53* and were thus definitely pathogenic. The fifth mutation was an in-frame deletion of one amino acid that was also judged to be pathogenic.
Conclusion: No obvious difference in the *TP53* mutation spectra was seen by 3D viewer, provided by IARC TP53 database, in patients with hereditary breast cancer compared to those with LFS. Therefore, we currently analyze the novel mutations by CRISPR technology.

PM12.140
Pilot study with TruSight Cancer genes panel in high risk patients at MMCI - Czech Republic

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Currently, more than 200 hereditary cancer syndromes has been described, but in most countries testing is very restricted with respect to the number of genes and the number of people tested.
As a retrospective research study the TruSight cancer panel (Illumina) - NGS panel targeting 97 cancer predisposition genes was used to analyze 50 high risk cancer patients with significant personal and family history of cancer without previously identified mutation in BRCA1, BRCA2, TP53, MLH1, MSH2, MSH6, APC or CDKN2A genes. All pathogenic mutations detected by NGS technology have been confirmed by Sanger sequencing.
Several deleterious (frameshift, nonsense) mutations were detected in ATM, ERCC2, BAP1, FANCI, PMS2, RECQL4 genes. Several very likely pathogenic missense mutations were detected in the ATM, FANCA, PALB2, BRIP1, MUTYH, PMS2, MEN1 and SDHB. These mutations affect highly conserved domains and were confirmed to affect protein function by available functional assays, or were confirmed to be pathogenic as a Parent #2 allele in a serious recessive disease as Ataxia telangiectasia or Fanconi anemia. Majority of missense variants detected in other genes remain to be classified by the

clinical relevancy in the future. Intermediate or low penetrance variants are of limited clinical utility.
The value of panel genetic testing in high risk individuals with cancer provides important information with respect to the cause and optimal treatment of the current cancer, and the risk and optimal management of future cancer.
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PS12.141
Evaluation of anti-tumor effect of combined use of thymoquinone with mitoxantrone in YKG1 glioma cells

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Introduction: Since thymoquinone is isolated from Nigella sativa, its antioxidant, anti-inflammatory and anti-cancer effects have been investigated. Thymoquinone causes decrease in cell viability in tumor cells and causes DNA damage and leads to apoptosis in tumor. Especially it has been found that it does not damage healthy cells and this makes it more important as therapeutic substance . In this study, we have proposed to reveal the role of thymoquinone on brain tumor cells by compairing with mitoxantrone and aimed to find more effective and less toxic treatment method.
Materials and Methods: YKG1 cell line has been thawed and proliferated. Main stock of thymoquinone was prepared in DMSO solution. Various concentrations of thymoquinone (T1:40 uM, T2:80 uM and T3:160uM) were prepared from main stock by diluting in DMEM solution. The cells were exposed to thymoquinone with these doses. Additionally the cells were exposed to 4 different doses of mitoxantrone (M4:0.5ug/ml, M3:0.05ug/ml, M2:0.005ug/ml and M1:0.0005ug/ml). Finally the cells were treated with both of mitoxantrone and thymoquinone series together.
Results: Antiproliferative effect of thymoquinone was seen only at T3 doses while both M3 and M4 doses of mitoxantrone showed toxic effect to the cells when they were applied individually. On the other hand, when combined use of mitoxantrone and thymoquinone were done, a significant antitumoral effect was seen in T2M4 combination.
Conclusions: The effect of T2M4 combination on the cell suppression was almost twice in M4 mitoxantrone alone application. We thought this result shows synergistic effect of thymoquinone with mitoxantrone.

PM12.142
Analysis of polymorphisms and epidemiological factors in selected genes coding for interleukins in women with uterine leiomyomas

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Several interleukins has been known to promote tumorogenesis in different tissues. Uterine leiomyomas (ULM) are the most prevalent pelvic tumors in women and it has been shown that genetic factors contribute to development of ULM. The aim of our study was to analyze SNPs in interleukins IL12RB1, IL12B and IL23R, which play an important role in immune response and to correlate genotypes with clinical data of patients with solitary and multiple leiomyomas. In our study 169 Slovenian ULM patients and 49 controls were included. We found significantly lower frequency of genotype AA for SNP rs11575934 (IL12RB1) in patients with solitary leiomyomas (39,0%, p=0,037) and multiple leiomyomas (45,9%, p=0,036) compared to healthy controls (62,5%). The results suggest that IL12RB1 (rs11575934) AA genotype is protective factor for developing ULM in Slovenian women for both forms of ULM. The analysis of epidemiological factors compared with allele frequency showed significantly higher number of pregnancy (p=0,048) and parity (p=0,004) in women with multiple leiomyomas with A allele for SNP rs11575934 (IL12RB1). Our results suggest that IL12RB1 gene polymorphism might be candidate genetic marker for prediction of susceptibility to leiomyoma and also suggest a link between genotype and the development of one of the forms of leiomyomas in the presence of specific epidemiological factor.

PS12.143
BAP1 germline mutations in Finnish uveal melanoma patients

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Aim: Germline mutations in BRCA1 associated protein-1 (BAP1) predispose to uveal melanoma and other cancers. Here, we estimated the frequency of BAP1 germline mutations in Finnish uveal melanoma patients.
Methods: We collected genomic DNA from 149 patients treated between 2010 and 2013. In addition, we identified 10 families each with two uveal melanoma patients. We were able to collect DNA from 13 members of uveal melanoma families. All 17 exons of the BAP1 were sequenced in total of 162 patients.
Results: We identified two pathogenic heterozygous BAP1 mutations: a donor splice site mutation in a highly conserved region in intron 2 in a sporadic patient and a frameshift insertion in exon 14 in three familial patients. The frequency for sporadic mutation is 0,67% (1/149, 95%CI <0,0001 to 0,04). Including the three familial mutation carriers to calculations, the frequency of BAP1 mutations is 2,5% (4/159, 95%CI <0,0076 to 0,0651). The insertion was found in two, possibly distantly related, families. The mutations were not present in ExAC database including 3325 Finnish controls from the SISu project.
Conclusions: BAP1 mutations predispose to uveal melanoma also in Finland. Mutations explained uveal melanoma in one sporadic patient and in two families of ten. Further studies are needed to study the genetic background of uveal melanoma in Finland using genome wide analysis strategies.
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PM12.144
Genetic background of unilateral/bilateral Vestibular Schwannoma (VS)

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A vestibular schwannoma (VS) (acoustic Neurinoma) is a benign tumors originated from Schwann cells (SC) of the vestibular nerve and located in the cerebello pontine angle or the inner auditory canal. The tumor results from an overproliferation of SC; these cells wrap themselves around nerve fibres, often causing gradual hearing loss, tinnitus and dizziness. It can also affect with the facial nerve causing paralysis by compression. Early detection of the tumour is sometimes difficult, because the symptoms may be subtle and may not appear in the beginning. There are two types of VS: Bilateral and unilateral. Until now there is only one single gene known for bilateral VS, that leads to neurofibromatosis type 2 (NF2). Unilateral VS account for approximately 8 % of all cranial tumours. The exact cause of unilateral VS is unknown, most occur spontaneously. Our study group consists of 144 patients (from 139 independent families) of which 23 have a bilateral VS (NF2), 119 unilateral VS and 2 with an initial suspicion of VS. All patients were clinically clearly defined. Genetically we performed a mutation analysis on the NF2 gene at the genomic and tumor level. So far we identified different missense/nonsense and splice mutations in NF2 gene, on the genomic level and in some cases exclusively in tumor cells, which all lead to a loss of function of the gene product Merlin and the appearance of VS. In addition to somatic changes and mutations there are possible indications of a genomic predisposition to unilateral Vestibularisschwannome.

PS12.145
Complex chromosomal aberrations with gain of 1q32.2 and 3q25 to 3qter and deletion of 17p in relapsed Waldenström's macroglobulinemia

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The genetic abnormalities in Waldenström's macroglobulinemia (WM) are relevant for the differential iagnosis of related B-cell malignancies as well as for risk stratification in WM patients. We present a case of WM progressed to therapy-related acute myeloid leukemia, showing complex chromosomal abnormalities found during tumor progression, including gains of 1q and 3q, deletion of 17p, and monosomies 18 and 20.
In 2002, our patient at 53-years old was diagnosed with extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue by par-

otid gland biopsy. She was treated with local radiotherapy and achieved complete remission. In 2010, she was diagnosed with WM and treated with 6 cycles of a fludarabine, mitoxantrone, and dexamethasone regimen. But in 2011, BM biopsy revealed involvement of LPL (52.2% of ANCs), and the karyotype was normal. In 2013, Microarray and conventional karyotyping from BM involvement sample revealed 45,XX,add(3)(q27),-18,-20,+mar[10/25].arr[hg19] 1q32.2(207,319,569-207,838,127)x3,3q25.1q27.1(150,744,552-183,300,655)x2~3,17p13.3p11.2(18,900-16,450,821)x1~2,18p11.32p11.21(136,304-14,144,899)x1~2,18q21.2q23(51,396,071-77,611,182)x1~2,20q11.21q13.33(31,027,513-62,532,060)x1~2. The *MYD88* L265P and *ARID1A* mutation, identified as a recurring mutation in WM, were not detected in our case. In December 2013, she was diagnosed with t-AML and treated with decitabine, but died in 2014.

Deletion of 17p13, recurrent abnormalities in WM and associated with disease progression, was found in our case in the advanced stage suggests that the aberrations occur as a secondary event. Among these duplicated 3q region in our case, *KLHL6* (kelch-like protein 6) was identified as a target of somatic hypermutation in CLL from whole-genome sequencing experiments. Close monitoring of cytogenetic abnormalities during WM treatment would be needed for possibility of therapy-related leukemogenesis.

PM12.146

Y chromosome loss in blood is associated with colorectal and prostate cancer

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Although age-related loss of chromosome Y (LOY) in normal hematopoietic cells is a well known phenomena, the phenotypic consequences of LOY have been elusive. Recently, LOY was found in association with smoking, shorter survival and different types of cancer. It was suggested that LOY in blood could become a predictive biomarker of male carcinogenesis.

The aim of our study was to investigate the association of LOY in blood with colorectal (CC) and prostate cancers (PC). For this purpose we have analyzed DNA samples, isolated from peripheral blood of 102 CC patients (mean age 59.8±13.2), 70 PC patients (mean age 68.8±8.0) and 93 healthy control males (mean age 65.8±16.6). The methodology included multiplex quantitative fluorescent (QF) PCR of chr.X/chr.Y (amelogenine gene), chr.1/chr.Y and chr.3/chr.X homologous sequences followed by automatic detection and analysis on ABI 3500 Genetic Analyzer. The mean Y/X ratios were significantly lower in CC (0.881±0.15; p=2.32x10-9) and PC patients (0.941±0.06; p=0.00012) in comparison to the controls (1.015±0.15). Substantial LOY (determined as Y/X ratio < 0.70) was significantly more frequent among CC patients (10/102 or 9.8%) in comparison to the controls (2/93 or 2.15%; p=0.0352) and PC patients (0/70 or 0%; p=0.0059). Multivariate regression analysis adjusting for tumor localization, stage and Y chromosome lineage was unable to link substantial LOY to certain colorectal cancer subgroups.

Our results support the recent findings of association of LOY in blood with carcinogenesis in males. They also show that LOY is more significant among colorectal cancer than prostate cancer patients.

PS13.01

A 17p13.3 microduplication including the PAFAH1B1 and YWHAE genes resulting from a maternal balanced 17; 3 translocation

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Introduction: 17p13.3 is a chromosomal region of genomic instability due to extensive repetitive sequences. In literature, microdeletions and microduplications have been described leading to different clinical phenotypes.

Materials and Methods: We describe the case of 2-year-old girl with a microduplication of the MDS critical region, involving the *PAFAH1B1* and *YWHAE* genes. The microduplication is resulting from maternal balanced translocation. The patient presented with delayed psychomotor development and dysmorphic featurs. She is born of inbreed marriage at the 2nd level, she had a brother and a sister died at one year old due to lissencephaly. His MRI is normal. To explore the genetic level, R-band karyotype and Fluorescence *in Situ* Hybridation (FISH) and Array CGH are performed.

Results: We conducted a FISH using a probe specific for the *LIS1* gene en 17p13.3 and telomeric probes en chromosomes 3, which showed a 17p13.3 duplication and subsequently duplication of LIS1 gene and 3p26 deletion for many reasons: the deletion of 2.9 Mb on chromosome 17 and the duplication of 3.6 Mb on chromosome 3 in her sister detected by CGH Array, the maternal translocation between the chromosome 3 and the chromosome 17.

Conclusions: Phenotypic comparison with the other patients found in the literature revealed common phenotypic characteristics such as mild to moderate developmental delay psychomotor and facial dysmorphism including high forehead and small mouth. Frequently, MRI of patients shows agyria, pachygyria···etc. However, our MRI patient returned normal but this does'nt mean that the duplication of the *LIS1* gene causes neuronal migration disorders.

PM13.02

A new microdeletion involving 1q32.1 region identified by high-density chromosomal microarray analyses

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Background: To our best knowledge, only one case with a deletion involved 1q32.1 characterized by aCGH has been previously reported. **Methods and Results:** Here, we described a 16 year-old female patient with mild facial dysmorphic features, intellectual disability (ID) and marfanoid habitus (MH). CytoScan HD array analyses identified a 1q32.1 interstitial microdeletion of 1.87 Mb (hg19:200,852,854-202,726,877). To date, only one case with 1q32.1 microdeletion that overlaps with our patient has been reported. We identified 3 additional cases with similar deletions annotated in the databases [DECIPHER (259811-288679) and ISCA (nssv575720)]. Interestingly, Olson *et al* (2012) described two patients with similar microduplications at 1q32.1 associated with neurodevelopmental delay that overlap with the four cases mentioned above. Excluding one case (259811) all had neurodevelopmental delay (NDD) or intellectual disability (ID). Our patient presented the shorter genomic imbalance and the genotype-phenotype correlation allowed us to identify a minimal overlapping region (MOR) that includes 31 RefSeq genes. We analyzed which of these genes are expressed in the brain and the probability of haploinsufficiency (HI) of this genomic region, resulting *KDM5B* as the best candidate for the NDD/ID. *KDM5B* is a histone lysine demethylase which play a role in neuronal differentiation. Neurodevelopment genetic disorders involving alterations in this mechanism have been described. **Conclusions:** We propose that the microdeletion of 1q32.1 is a rare pathogenic CNV and that the MH is an unspecific finding of this genomic deletion. Finally, we suggested that the (HI) of *KDM5B* gene could be involved in the NDD/ID.

PS13.03

Array Based Comparative Genomic Hybridization Applying for Multiple Congenital Anomalies, Developmental Delay/Intellectual Disability, Autism Spectrum Disorders, and Seizure disorder in Taiwan

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Array Based Comparative Genomic Hybridization (array-CGH) has been confirmed to increase the diagnosis of unexplained multiple congenital anomalies (MCA), developmental delay/intellectual disability (DD/ID), autism spectrum disorders (ASD), and seizure disorder (SD). The purpose of this study was to provide related information in Taiwan because of the limited statistic data before. We retrospectively collected the array-CGH results and medical records of 145 patients with MCA, DD/ID, ASD, or SD at Mackay Children's Hospital from June 2010 to December 2014. Blood samples were analyzed by Affymetrix GeneChip Genome-Wide Human SNP array 6.0 with a resolution ranging from 100 Kb to 200 Kb and NimbleGen ISCA plus Cytogenetic Array with a resolution of 30 Kb. Of the 145 patients in this study, 79 (54%) males and 66 (46%) females were enrolled and the median age was 5.16 years. The proportions of MCA, DD/ID, ASD, and SD were 68% (99/145), 43% (62/145), 6% (8/145), and 9% (13/145), respectively. Some patients had more than one clinical feature. Copy number variants were detected in 57 patients, including microdeletions in 37, microduplications in 8, and two different rearrangements in 12 patients. Among these 145 patients, 99 (68%) also had G-banded karyotyping examination. The positive results of array-CGH and G-banded karyotyping were found in 47 patients (47%) and 9 patients (12%), respectively. Our results further demonstrated the

higher diagnostic yield in the assessment of patients with unexplained MCA, DD/ID, ASD, and SD. The use of array-CGH also strongly supports as the first-tier cytogenetic test in place of G-banded karyotyping.

PM13.04
Array-CGH analysis in a large cohort of patients with intellectual disability and/or congenital malformations

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Array-CGH analysis has improved the detection of pathogenic imbalances in patients with intellectual disability and congenital malformations and demonstrated that copy number variations (CNVs) are a major source of genetic diversity between normal individuals. Recurrent CNVs are usually caused by non-allelic homologous recombination (NAHR) between LCRs, while several microhomology-mediated repair mechanisms including Non Homologous End Joining (NHEJ) and Fork Stalling and Template Switching (FoSTeS) have been proposed to explain the etiology of non-recurrent CNVs.
In this study we evaluated the frequency of CNVs in a cohort of 1051 patients referred to our institution because of intellectual disability, developmental delay, autism or multiple congenital anomalies. CNVs breakpoints were analyzed to identify sequence microhomologies that may have mediated the rearrangement. We also assessed whether the mechanism of formation or the CNV pathogenicity were associated with the inheritance (*de novo* or inherited anomaly), the type (deletion or duplication) or the size of the imbalance.
Array-CGH analysis detected at least one pathogenic or likely pathogenic CNV in 15.8% of patients and the anomalies were more likely deletions and CNVs arisen *de novo*. Most of the imbalances were caused by microhomology-mediated mechanisms (74.2%) but no significant differences were observed with regard to the type of CNV or the pattern of inheritance.
Moreover, while most of the NAHR-mediated CNVs had dimensions ranging between 1Mb and 5Mb and were concentrated in few chromosomes, microhomology-mediated CNVs had variable size and their frequency was statistically positively correlated with the dimensions of chromosomes.

PS13.05
Reciprocal autosomal balanced translocation and Yq duplication in an azoospermic man

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In male infertility sperm counts usually range from oligospermia to azoospermia. Azoospermia is defined as the absence of live spermatozoa in semen, and may be obstructive or nonobstructive depending on the presence of blockage of the tubules or ducts. Chromosomal rearrangements are a recognized cause of reproductive failure in males, with a significantly higher rate of cytogenetic anomalies observed in azoospermic (13.7%) than in oligospermic men (4.6%). In addition, Y chromosome microdeletion is a major genetic cause, specifically involving azoospermia factor regions (AZF). However, duplications involving AZF at Yq have been described both in infertile and fertile men. Here we report the case of an azoospermic man with a reciprocal autosomal balanced translocation and Yq duplication. Karyotype in blood lymphocytes was 46,XY, t(6;13)(p12;p13) and array genomic hybridization was performed to search for copy number alteration at breakpoints. No abnormalities were found in these regions; although, a 968kb duplication (24017591-24985599, hg19) at Yq11.223 was detected, including part of AZFc region and twelve genes, including *RBMY1*, which is expressed during spermatogenesis. As the role of increased expression of some genes inside the duplicated AZF region is controversial and Yq duplication is inherited from his normal father, can be hypothesized that the translocation led to meiotic impairment or (and) disruption of an autosomal gene involved in gametogenesis. Cases of male infertility in carriers of translocations involving chromosomes 6 or 13 have been described. This case reinforces the importance of cytogenetic together with molecular characterization. Supported by Fapesp (2011/23794-7 and 2012/10071-0) and CNPq (304455/2012-1).

PS13.07
Molecular analyses of the effects of clock gene SNPs on the Biological Clock

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Circadian clocks coordinate physiology and behavior with the 24 h solar day to provide temporal homeostasis with the external environment. The mammalian circadian clock is based on a transcription-translation feedback loop in which CLOCK and BMAL1 proteins act as transcriptional activators, CRYs and PERs proteins repress CLOCK/BMAL1. SNPs in the clock genes are associated with many physiological disorders like sleep disorders, metabolic disorders, and infertility. However, the effects of SNPs on biological clock and the related disorders are not well understood.
In the present study, the effects of SNPs on core clock protein's function and thus, on the biological clock was investigated. For each gene 8-10 SNPs that placed in functional domains like HLH and PAS1-2 were chosen based on the SIFT and PolyPhen bioinformatics tools. Gene variants were created by site-directed mutagenesis. The effects of the SNPs were examined by looking the interaction between clock proteins, and CLOCK/BMAL1 dimer driven transciption. We performed preliminary studies with Bmal1 gene. Based on lusiferase analyses, p.Arg84Cys (COSM296154) or p.Leu196Gln (rs112626431) variation result in decrease in transactivation capacity of BMAL1/CLOCK dimer. Additionally, CRY2/PER2 dimer has different inhibition effect on variant BMAL1/CLOCK dimer than wild type BMAL/CLOCK dimer.
As far as we know, this study will be the first study investigating the effects of SNPs resulting in amino acid changes in clock proteins at molecular level. The findings of the study will enable us to investigate the relation between investigated candidate clock SNPs and clock related diseases such as cancer, metabolic disorders, diabetes, and obesity. This study was supported by TUBITAK 114Z879 grant.

PM13.08
Characterization of the complex breakpoint junction of a CFTR gene deletion with discordant MLPA and qPCR findings

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The reported incidence of rearrangements of the CFTR gene varies from 2-20% in affected probands with multiple underlying mutation mechanisms proposed. More recent investigations reveal that sequences at the breakpoints of CFTR rearrangements can reflect more complex patterns of breakage and rejoining. As part of our provincial newborn screening program, a male infant was found to be heterozygous for the deltaF508 variant (CFTR c.1521_1523delCTT). Subsequently he had an abnormal sweat chloride test and full gene analysis for sequence variants and gene rearrangements of the CFTR gene was initiated. By MLPA he was found to carry a deletion of exons 17-20. Analysis by quantitative real-time PCR could only confirm the presence of a deletion of exons 18-20. Sequencing of the deletion breakpoint revealed a complex rearrangement that confirmed the loss of exons 18-20, but also found inserted at the breakpoint junction an inverted portion of intron 18 (119 bp) joined to a GA dinucleotide and then an inverted portion of exon 17 (66bp) (CFTR c.2908+353_c.3368-1088delins(c.2988+1022_c.2988+1141inv:GA:c.2717_c.2782inv)). The abnormal MLPA result for exon 17 is hypothesized to be due to the duplicated portion of exon 17 acting as a "sink", binding the MLPA probe whilst not permitting amplification. Microhomologies (2-8bp) and short repetitive elements at or near the breakpoints, the size of the rearranged sequences, and their proximity to one another suggest that a replicative-based mechanism such as fork stalling and template switching may underlie this rearrangement. To our knowledge this represents the most complex CFTR rearrangement reported in the literature.

PS13.09
Three cases of complex X chromosome rearrangements that appear to have been mediated by chromoanasynthesis

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In recent years there have been a number of reports of chromothripsis-like events in patients with developmental delay and congenital abnormalities. Chromothripsis is believed to involve an initial trigger which causes chromosome shattering and repair by non-homologous end joining (NHEJ). However, the vast majority of reported cases of constitutional chromothripsis have copy number profiles that more closely resemble a replication-based event distinct to chromothripsis called chromoanasynthesis (Liu et al., 2011). The key difference between the two processes is that whilst chromothripsis is characterised by loss and retention of heterozygosity, chromoanasynthesis

can also result in gains, including duplications and triplications. We report three patients, two male and one female, each with a developmental disorder, who were referred for array CGH analysis. They were all shown to have multiple duplications on the X chromosome. This has resulted in the partial duplication of the MECP2 gene in one patient and the partial duplication of the CDKL5 gene in another. The presence of multiple copy number gains clustered on a single chromosome is consistent with chromoanasynthesis. No similar complex rearrangements involving an autosome chromosome have been detected in our laboratory. To the best of our knowledge, these are the first patients reported with multiple copy number gains on the X chromosome.

PM13.10
Chromosomes in a genome-wise order change the landscape of genetics

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Although a non-random distribution of chromosomes was suggested already in the early days of human cytogenetics it is commonly accepted by the majority of cytogeneticists that chromosomes in a metaphase spread are arranged in a completely random way. Since the observation of the bilaterally symmetric distribution of DNA and chromosome specific fluorescence in situ hybridization (FISH) signals in leukocytes in 1990, we demonstrated in a series of publications the genome-wise organization of chromosomes in human and murine cells. In other words, the maternal chromosomes are tethered to one centriole while the paternal chromosomes are connected to the other. Between 1956 and 1991 similar observations were made in human, insect and plant cells, still these observations were widely ignored. As a rule, rather than an exception, we found this genome-wise haploid order of chromosomes in a variety of samples from different human and other mammalian tissues; in numerically aberrant human karyotypes and samples with small supernumerary marker chromosomes (sSMC). Detailed analysis of the 3 cases with SMC and uniparental disomy showed that there is a defined nuclear architecture in metaphase allowing bilateral organization of the two haploid sets of chromosomes organized in haploid groupings corresponding to parental origin. Thus, we may have to refine the terms "Comparative Genomic Hybridization" or "Loss of Heterozygosity" by specifying the involvement of the maternal and/or paternal genomes. Supported by Carl Zeiss MicroImaging GmbH, Germany.

PS13.11
PMS2 inactivation by a complex FoSTeS-mediated rearrangement involving the inverted 100-kb duplicon on 7p22.1 and a HERV retroelement

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Biallelic PMS2 mutations are responsible for more than half of all cases of constitutive mismatch repair deficiency (CMMRD), a recessively inherited childhood cancer predisposition syndrome. The 3' region (exons 9-15) of the PMS2 gene is located in a 100-kb sequence that is duplicated ~0.7-Mb centromeric in inverse orientation on 7p22.1. Sequence exchange, primarily homologous recombination (HR) with crossover between the 100-kb duplicons, is thought to be responsible for the known genomic inversion polymorphism of the 0.7-Mb sequence intervening the duplicons. PMS2 transcript and MLPA analysis in a new CMMRD patient rendered results that were best explained by a genomic rearrangement separating the N-terminal from the C-terminal region of the gene. GenomeWalker analysis and 3'RACE PCR uncovered the breakpoints of a complex rearrangement that is characterized by a ~918-kb inversion that exceeds the known inversion polymorphism by ~220-kb. This inversion was further associated with a duplication of ~100-kb of the inverted region. Fork stalling and template switching/microhomology-mediated break-induced replication (FoSTeS/MMBIR) is the most parsimonious mechanism by which the rearrangement truncating the PMS2 gene could be explained. These findings support the hypothesis that inverted segmental duplications can not only mediate the formation of simple inversions by HR but also more complex structural rearrangements. It further suggests that complex rearrangements with copy number alterations could be hidden among the genomic inversion on 7p22.1.

PM13.12
Whole exome sequencing detects p.Q155X mutation in CRYBB2 gene; evidence of heterogeneity genetic in a family with autosomal dominant pulverulent cataract

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Congenital cataract is an entity clinical and genetically heterogeneous with high penetrance and represents 10% of a treatable cause of childhood blindness. Thirty percent of the cataracts are heredity with the nonsyndromic autosomal dominant form. About 30 loci have been associated to congenital cataract. CRYBB2 gene belongs to β crystallin family together with 6 genes (4 CRYBA and 2 CRYBB) on several chromosomes and is related to congenital cataract. In the present study, we analyzed a Mexican family affected by autosomal dominant pulverulent cataract in four generations through whole exome and identified the cC475T (pQ155X) mutation on CRYBB2. Nonsense cC475T (pQ155X) mutation has been reported previously from five different countries and predicts a stop codon on Q155 losing 51 aminoacids and four Greek key domain in the C-terminal region of the CRYBB2 protein. Gene conversion seems to be the cause of this type of cataract cataracts. This is the first Mexican family with pulverulent cataract associated to a pQ155X mutation. This data shows evidence of heterogeneity genetic in autosomal dominant pulverulent cataract but a consistent presence of this morphology when CRIBB2 is affected. Exome sequencing comes more as a powerful tool for the detection of genetic origins of cataracts due to genetic and clinical heterogeneity in this type of disease

PS13.13
Sequencing of CFTR gene at the patients with classical and atypical forms of cystic fibrosis

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Cystic fibrosis (CF) is a common autosomal recessive disease caused by mutations in the CFTR gene encoding for the cystic fibrosis transmembrane conductance regulator protein. The CFTR gene is characterized by an extremely large number of mutations (more than 1900). To date, after the routine genetic testing including the analysis of the most frequent mutations of CFTR gene about 30% of mutations remain unidentified. We studied CFTR gene in cohort of 50 CF patients with one or both unknown mutations. We performed sequencing to characterize the mutations in coding regions, promoter and splice sites in CFTR gene. To exclude large gene deletions/insertions the method of MLPA was used. 71% of patients including in this study have classical form of CF and 29% - atypical mild form of disease. We identified 5 rare mutations with quite high frequency in Russian population (3849+10kbC/T, E92K, 489+1G>T, Ser1231ProfsX4, L1335P) that aren't included in panel of mutations for routine CF testing in Russia. Three novel nonsynonymous mutations (E92A, K1468R, Ile1328Lys), one deletion (3816_3817delTG) were identified. One patient had a large deletion from 3 to 10 intron (CFTR40kbdel). In our study mutations of CFTR gene were found in 85,6% of CF chromosomes. Among patients with classical form mutations were identified in 94% of CF chromosomes whereas among patients with atypical form - only in 61%. These results prompt studies of noncoding regions of CFTR, including intronic regions, potentially involved in the regulation of gene expression at the patients with atypical form of CF.

PM13.14
DNA repair factors are upregulated following Dental Cone Beam CT irradiation

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The Dental Cone Beam CT (DCBCT) has been specifically developed for dental use and offers a volume 3-dimensional imaging similar to medical CT but with significantly lower radiation exposure of the patient. Still, ionizing irradiation is a source of DNA damage.
The aim of this study is to determine DCBCT irradiation consequences at molecular level by studying the expression of factors involved in DNA Damage Response (DDR) pathways.
HEK293 cells, used as experimental material, were placed into a house-

made phantom device, manufactured to mimic as possible the respective doses an adult tissue would receive. Cells were irradiated using a NewTom DVT 9000, DCBCT. DDR response was monitored by detecting γH2AX, p53, Rad51, p21^{waf1} and BRCA1 proteins in two time points following irradiation, by immunofluorescence and western blot. The respective mRNA levels were also studied.
Characteristic foci of phosphorylated γH2AX, a marker of ds DNA damage, were clearly detected in HEK293 cell nuclei, in just half an hour after irradiation. In accordance, altered protein levels of key molecules involved in DNA repair such as BRCA1 and Rad51, were also observed. More specifically, BRCA1 protein was significantly induced in at least half an hour after irradiation, while Rad51 protein levels were higher than normal 48hours following irradiation. No significant modification was observed in p53 and p21^{waf1} protein levels.
In conclusion, DCBCT irradiation of HEK293 cells results in at least temporary modification of molecules involved in DNA damage detection and repair. Based on these results, a more concrete evaluation of DCBCT irradiation risk assessment at the molecular level is required.
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PS13.15
Assisting research into human embryonic and fetal development

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The Human Developmental Biology Resource (HDBR) is a unique resource funded by the MRC and Wellcome Trust. It provides human embryonic and fetal tissue for gene expression studies related to congenital disease, including birth defects and inherited metabolic disorders. Use of the material should particularly illuminate developmental gene expression underlying aspects of functioning that characterise humans as opposed to lower animals (e.g. higher brain function, language). This research is essential if we are to introduce new methods for prevention of congenital defects and develop an improved understanding of "what makes us human".
The HDBR has Tissue Bank ethics approval for the collection, storage and distribution of material between 4 and 22 weeks of gestation. A significant proportion of the HDBR material is karyotyped and chromosomally normal material is provided for research but karyotypically abnormal material can also be provided on request. The HDBR's material can be used to generate cell lines, stem cells, protein, RNA and DNA. The HDBR can also provide cDNA from embryonic tissue for gene expression analysis. In addition, paraffin wax and frozen sections of embryos and early fetuses are available for in situ hybridisation and immunohistochemistry. Individuals wishing to use human and fetal tissue do not need to apply for local ethical permission as the HDBR ethics covers distribution of tissue to end users. Human embryonic and fetal tissue is provided free to registered users of the HDBR.
Further information can be found on our web site www.HDBR.org.
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PM13.16
14q32.3 deletions: molecular findings and genotype-phenotype correlations in two cases

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14q32.3 deletions are rare chromosome abnormalities. Nonetheless, studying genomic rearrangements by array CGH has shown 2 cases out of 350 to demonstrate 14q32.2q32.33 and 14q31.31q32.33 deletions. Deleted regions spanned 6,26 Mb at 14q32.2q32.33 and 4,83 bp at 14q32.31q32.33, involving 386 and 268 genes, respectively. The former case also demonstrated mosaic 14q32.13q32.2 duplication (4,53 Mb) spanning 38 genes and non-mosaic 14q32.2 duplication (1,87 Mb) spanning 24 genes. We speculate that duplications flanking the deletion are likely to contribute to the formation. The first case presented with microcephaly, structural congenital myopathy, spinal defects, congenital heart defect, short stature and failure to thrive. The second patient presented with severe developmental and speech delays, autism, hyperactivity, microcephaly, mild congenital heart defect and bilateral nephroptosis. To assess molecular basis of phenotypes, we performed bioinformatic analysis. We found that DYNC1H1, HSP90AA1, TECPR2, TRAF3, CDC42BPB, CKB, KLC1, C14orf2 and PACS2 are candidate genes for neuropsychiatric abnormalities. CRIP2 and ADSSL1 are candidate genes for congenital heart defect. ZFYVE21, PPP1R13B, JAG2 and NUDT14 are associated with myopathy, spinal and kidney defects as well as contribute to immunological problems reported in these cases. The present study demonstrates that application of high-resolution array CGH analysis coupled with bioinformatics is able to shed light not only on gene misbalance, but also on formation mechanisms as well as molecular/cellular and phenotypic outcome of genomic variations through gene-centric genotype-phenotype correlations. Supported by a grant from the Russian Science Foundation (project №14-15-00411).

PS13.17
Functional characterization of congenital GPI-anchor deficiencies reveals substantial differences in the expression profiles of GPI-anchored substrates

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Glycosylphosphatidylinositol (GPI)-anchor deficiencies represent a phenotypically highly variable and diverse new class of congenital disorders of glycosylation. Over the recent years pathogenic mutations have been identified in 12 genes of the GPI-anchor synthesis and maturation pathway. There are certain features that are shared by most patients with a GPI-anchor deficiency such as intellectual disability or epilepsies. However, other features such as a characteristic facial gestalt, certain organ malformations or abnormalities in routine laboratory parameters, allow the delineation of distinct syndromes (Mabry syndrome, CHIME syndrome, MCAHS1-3).
With more than 300 known GPI-anchored proteins (GPI-AP), we hypothesized that the differences in the phenotypic landscape of GPI-anchor deficiencies might also be due to mutation specific changes in GPI-AP expression. We established a comprehensive flow cytometric protocol comprising 10 different GPI-anchored markers for different types of cell culture. We screened a cohort of 20 patients with pathogenic mutations in 5 different genes of the GPI-anchor synthesis and maturation pathway.
We found characteristic flow cytometric expression patterns for GPI-anchored markers for each analyzed gene. So far it has been assumed that any impairment of GPI-anchor synthesis would affect all GPI-APs uniformly. Thus our results are an unexpected finding and suggest that there might be a correlation between certain phenotypic features and the absence of certain GPI-APs.

PM13.18
Tissue distribution pattern of SMS1 protein implies active post-transcriptional regulation

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Sphingomyelin synthase 1 (SMS1) catalyses the synthesis of sphingomyelin and diacylglycerol from phosphatidylcholine and ceramide in eukaryotic cells. Sphingomyelin forms membrane rafts, which are important in membrane sorting and transport of proteins and in receptor signalling. The consumption and production of important regulators of cell processes such as ceramide and diacylglycerol, respectively, link SMS1 function to the processes of membrane transport, cell proliferation and apoptosis. We have previously determined the structure of the SGMS1 gene encoding this enzyme and a number of its alternative transcripts. Here, we present a study of the expression of the full-length SMS1 protein and the sum of the alternative transcripts encoding this protein in human tissues. Abundance of SMS1 protein was detected using immunodetection. SMS1 abundance was normalized to the total protein amount. Abundance of SGMS1 gene coding transcripts was detected using real-time PCR. The amounts of the transcripts of SGMS1 gene were estimated relative to the average mRNA level of housekeeping genes (LDHA, GAPDH and RPL3). The highest SMS1 protein expression level was detected in the kidney; low levels were observed in the placenta and the lymph node. Transcripts encoding the full-length SMS1 were most abundant in the placenta, minimal level were observed in liver. Thus, the SMS1 protein and mRNA levels in tissues differed significantly and were not correlated. Computer sequence analysis of the SGMS1 gene mRNA has revealed many binding sites of miRNA associated with Argonaute proteins in coding region and 3'UTR. We imply the active post-transcriptional regulation of SGMS1 gene expression.

PS13.19

Dosage effect of common trisomies in humans

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Chromosomal trisomies represent a significant share of morbidity and mortality in the paediatric population. We performed a comprehensive survey of transcriptional landscape in three potentially viable human trisomies (T21, T18, T13) to investigate whether global gene expression profile of these trisomies reflects a common homeostatic genome response or represents a specific gene-dosage effect of the underlying trisomy.

Transcriptomic analyses, comparing global gene expression profile between trisomic and normal cell line, using Agilent's Whole Human Genome Expression arrays, were performed in three groups: first and second comprised of the cultivated amniocytes with T21 and T18 (10 and 9 samples), while the third consisted of the cultivated cells of T13 chorionic villi (11 samples). For each group, a comparable number of tissue-matched normal cell line samples were used for comparison against reference expression profile.

Comparative analysis of differentially expressed genes in T21, T18 and T13 precipitated a subset of genes, differentially expressed in all three trisomies: WBP4 and PRPF8, involved in the mRNA splicing and PPID involved in protein folding. In addition, a co-expression network analyses demonstrated modular deregulation of gene clusters involved in cell division, RNA processing and transcriptional factors shared between T18 and T21, but not with T13.

Our data confirm that common trisomies induce transcriptome deregulation already in prenatal period. Moreover, we suggest that transcriptional alternations related to common trisomies reflect a genomic response associated with deregulation of genes involved in the cell cycle control, mRNA processing and important cellular signaling pathways. Further studies are needed to address the question of common homeostatic genomic response in common human trisomies.

PM13.20

Molecular characterization of a novel c.1092T>A splicing mutation in the IDS gene

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Pre-mRNA splicing is a fundamental step of gene expression by which introns are removed and exons are joined in pre-mRNAs to form the mature mRNA.

In this study, we have focused on the pathological c.1092T>A mutation in the *IDS* gene. Besides creating a *de novo* splice site, the mutation leads to the utilisation of multiple upstream cryptic splice sites and to the production of multiple aberrant transcripts in patient´s blood cells. This implies that a mutation likely changes a splicing regulatory element as well.

In order to elucidate the molecular mechanisms responsible for the altered splicing pattern we have used computational and minigene analyses. *In silico* predictions suggested that the mutation could lead to both disruption and creation of an exon splicing silencer, as well as of an exon splicing enhancer (ESE). Minigene analyses of the c.1092T>A mutation and of artificial deletion mutants indicated, that the c.1092T>A mutation indeed leads to the creation of a new splicing regulatory element. This outcome was further specified by results of an ESEdependent splicing assay that showed the creation of exon splicing silencer. Such silencer element might inhibit the use of the authentic splice site resulting in the observed complex splicing pattern. Our study highlights the importance of splicing regulatory elements analyses when examining splicing mutations. These sequences are relatively poorly defined and their alteration can lead to unexprected splicing patterns, with a significant impact on resulting phenotype.

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PS13.21

Methods of reprogramming to iPSC associated with chromosomal integrity and delineation of a chromosome 5q candidate region for growth advantage

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Induced pluripotent stem cells (iPSCs) have brought great promises for disease modeling and cell-based therapies. One concern related to the use of reprogrammed somatic cells is the loss of genomic integrity and chromosome stability, a hallmark for cancer and many other human disorders. We investigated the 16 human iPSC lines reprogrammed by non-integrative Sendai virus (SeV) and another 16 iPSC lines generated by integrative lentivirus for genetic changes. At early passages we detected cytogenetic rearrangements in 44% (7/16) of iPSC lines generated by lentiviral integration whereas the corresponding figure was 6% (1/16) using SeV based delivery. The rearrangements were numerical and/or structural with chromosomes 5 and 12 as the most frequently involved chromosomes. We present herein the karyotypic aberrations in the iPSC lines including a duplication on chromosome 5q13-q33 that restricts a candidate region for growth advantage. Our results suggest that the use of integrative lentivirus confers a higher risk for cytogenetic abnormalities at early passages when compared to SeV based reprogramming. In combination, our findings expand the knowledge on acquired cytogenetic aberrations in iPSC after reprogramming and during culture.

PM13.22

A deletion at 4p15.2 and disruption of PITX2 gene due to pericentric inversion in a patient with Axenfeld-Rieger syndrome and developmental delay

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Balanced rearrangements in patients with abnormal phenotype are often associated with cryptic microdeletion/microduplication or disruption of gene at the breakpoint. We report on a 2-year-old patient, a female, referred for genetic testing due to developmental delay and clinical features specific to Axenfeld–Rieger syndrome. It is long known that *PITX2* gene mutations, deletions or translocations involving *PITX2* or its surrounding genotypic landscape cause Axenfeld–Rieger syndrome.

Conventional karyotype of the patient revealed the presence of *de novo* pericentric inversion – inv(4)(p14q21~25). Array-CGH (1M) was performed and 2.4 Mb interstitial deletion at 4p15.2 was detected. FISH analysis using BAC FISH probes, RP11-118N21 (4p15.2) and RP11-313B13 (which encompasses the *PITX2* gene), confirmed the 4p15.2 deletion at the first breakpoint and indicated that the second chromosomal breakpoint at 4q25 was within or very close to the *PITX2* locus. The breakpoint at 4q25 directly interrupts *PITX2* gene structure or its regulatory elements and presumptively causes alteration of *PITX2* expression leading to clinical features of Axenfeld–Rieger syndrome. Haploinsufficiency of *DHX15* and/or *PPARGC1A* due to deletion at 4p15.2 may have contributed to manifestation of additional clinical features, as developmental delay, in the patient. To our knowledge, this is the first report of Axenfeld–Rieger syndrome, caused by pericentric inversion.

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PS13.23

Unsuspected diagnosis of Jacobsen syndrome in a patient with craniosinostosis surgically treated

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Partial deletions of the distal region of the long arm of chromosome 11 cause Jacobsen syndrome (JS). The breakpoints of deletion region occur around 11q23.3 and generally range from 7 to 20Mb. Occurrence of JS is 1 in 100,000 newborns with a female predominance. The cardinal clinical findings are growth retardation, intellectually disability, dysmorphism and thrombocytopenia/pancytopenia. Sometimes, systemic anomalies are found to be present. In general, the spectrum of the clinical manifestations depends on the size of the deleted region; however, a variable phenotype could be observed. Diagnosis is suspected on the basis of phenotype, but his must be confirmed by cytogenetic analysis or aCGH. The aim of the present study is to describe a patient with Jacobsen syndrome which underwent surgery to correct the craniosinostosis and had a heavy bleeding due to unsuspected diagnosis. Patient was a 18-months male, product of healthy, young and non-consanguineous parents. At birth, he presented dolichocephaly. At 5 months of age he underwent sagittal suturectomy presenting profuse bleeding. Physical

examination at 18 months of age revealed trigonocephaly, exotropia, reverse epicanto, high and narrow palate, micrognathia, right cryptorchidism and mild psychomotor retardation. Blood analysis reported normal platelet count with increased platelet volume. He again underwent surgery to correct craniosinostosis presenting hypovolemic shock. When the patient is referred to the Genetic Service it is detected a karyotype 46,XY,del(11)(q23) and an aCGH with a loss of 14.2 MB which corresponds to a Jacobsen syndrome. It is important to make an early diagnosis of patients with Jacobsen syndrome, in order to avoid complications during surgical procedures.

PM13.24
Karyotype is not dead (yet)!

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As array-comparative genomic hybridization (a-CGH) and high-throughput sequencing (exome) technologies have swiftly spread throughout the medical field, karyotype has gradually lost its leading role among genetic tests. For example, in case of a child with intellectual disability (ID), without any specific sign leading to a precise clinical diagnosis, several international guidelines recommend to start with a-CGH screening then go on with exome analysis. Generalized use of whole genome sequencing increases etiologic diagnoses rate up to 30% in case of ID. These technologies are definitely major steps in the etiological process of ID.
However, physicians have to deal with the lack of qualitative information of the genome. Especially, exome and a-CGH analysis fail to reveal chromosomal rearrangements because breakpoints are either located in introns or not associated with a gain or lost of genetic material. If theses quantitative technologies cannot easily identify chromosomal translocations or inversions which sometimes split a gene in two, karyotype does.
Here, we show 3 cases whose karyotype swiftly provided us the right diagnosis for a monogenic disease while gene molecular analysis had remained unsuccessful. When clinical diagnosis of a monogenic disease is suggested, the molecular analysis of the targeted gene may sometimes fail to identify the causal mutation. Then it could be very useful to carry out a karyotype in order to reveal a chromosomal rearrangement involving the targeted gene. If this gene is split in two confirmed by FISH, then the physician is not only able to confirm the causing disease but also to give the appropriate genetic counselling.

PS13.25
Investigating clinical impact and mechanism of formation of karyotypically balanced chromosomal rearrangements: the power of combining breakpoint mapping by mate-pair sequencing and copy number analysis by aCGH

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About 7% of karyotypically balanced chromosomal rearrangements (BCRs) are associated with developmental disorders (DD) due to gene or regulatory element disruption, cryptic imbalances on rearranged chromosomes and position effect. In this study we illustrate the power of mate-pair sequencing (MPS) and aCGH to investigate the clinical impact and to disclose the mechanisms of formation of BCRs in patients with DD. In our cohort of 43 BCRs (28 translocations, 10 inversions, five complex rearrangements), 180K aCGH revealed 32 likely pathogenic imbalances (2.2kb-6.6Mb) on rearranged chromosomes in 12 BCRs. Breakpoint mapping by MPS were performed in 27/43 BCRs. MPS expanded the number of breakpoints detected by karyotyping or aCGH from 114 to 162 (resolution <2kb). The 48 additional breakpoints were found in nine BCRs. The number of breakpoints/BCR ranged from 2-22; the five most complex BCRs (10-22 breaks) displayed hallmarks of *chromothripsis*. Gene disruption was observed in 25/27 BCRs: 10 BCRs disrupted known diseases genes or their regulatory region, and five others disrupted candidate genes. Overall pathogenic imbalances on rearranged chromosomes and/or disrupted known disease genes/regulatory regions were identified in 19/44 BCRs, proving the effectiveness of aCGH/MPS for genotype-phenotype correlations. Among 30 Sanger-sequenced breakpoint-junctions from 13 BCRs, five contained >45 nucleotide deletions; 19 breakpoint-junctions had <23 nucleotide insertions or deletions; six had no alterations. Micro-homology of 2-7 nucleotides were detected in 11/30 breakpoints-junctions; one breakpoint-junction had a 30 nucleotide homology. These findings implicate non-homologous (NHEJ) or microhomology-mediated (MMEJ) end joining mechanisms in the formation of these BCRs. Financial support: FAPESP.

PM13.26
Deep sequencing detects very low-grade somatic mosaicism in the unaffected mother of siblings with nemaline myopathy

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When an expected mutation in a particular disease-causing gene is not identified in a suspected carrier, it is usually assumed to be due to germline mosaicism. We first report very low-grade somatic mosaicism in ACTA1 detected only by deep resequencing using next-generation sequencer in an unaffected mother of two siblings affected with nemaline myopathy of neonatal form. We identified a novel heterozygous mutation in ACTA1, c.448A>G (p.Thr150Ala), in the affected siblings. 3D structural modeling suggested this mutation may affect polymerization and/or actin's interactions with other proteins. Autosomal dominant inheritance with either parent being either a germline or somatic mosaic was mostly expected. Sanger sequencing identified no mutation. Further deep resequencing of this mutation by next-generation sequencer identified very low-grade somatic mosaicism in the mother: 0.4%, 1.1%, and 8.3% in saliva, blood leukocytes, and nails, respectively. Our study demonstrates the possibility of very low-grade somatic mosaicism existing in suspected carriers, instead of germline mosaicism.
Drs. Yukiko K. Hayashi, Kazushi Miya, Masaaki Shiina, Kazuhiro Ogata, Ichizo Nishino are highly appreciated for their contribution to this work.

PS13.27
Non-identical twins: miR-96-5p and miR-96-3p expression during mouse development

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Introduction. We previously reported a deafness-causing mutation within the MIR96 microRNA gene, miR-96(+57T>C), which alters the correct maturation of the miR-96 precursor and also affects the seed region of miR-96-3p (also called miR-96*). Although the function of miR-96-5p in the inner ear has been highly investigated, the biological role of its partner strand still remains unclear.
Methods and Results. We analyzed the expression pattern of both miR-96-3p and -5p in the developing mouse embryo (from 10.5 days post coitum to the end of gestation), using specific LNA probes for in-situ detection. Although both mature miRNAs were co-expressed in several tissues (neural tube, somites, and developing ear) a different spatio-temporal distribution between the -3p and -5p was found. To understand which factors may influence miRNA strand selection, we are currently combining miRNA in-situ hybridization and immunohistochemical staining for proteins involved either in miRNA biogenesis or in RNA interference (e.g. Ago1-4, TRBP). Finally, candidate miR-96-3p targets were predicted and are being functionally validated by ex-vivo assays.
Conclusions. For the first time, we report the highly specific and regulated pattern of expression of miR-96-3p during mouse development, suggesting both distinct and common roles compared to its partner miRNA.
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PM13.28
UNCOMMON NUMERICAL CHROMOSOMAL ABNORMALITY: MOSAICISM 47, XY(+8)/ 47, XY (+21) Case report

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Introduction. Trisomy 8 is a rare numerical chromosomal abnormality characterized by the presence of additional chromosome 8. The complete form is fatal resulting in early death. Life expectancy in trisomy 8 mosaicism, in the absence of serious malformations is that of the general population. The incidence of trisomy 8 mosaicism is 1:25000-1:50000, more common in boys (5:1) although the explanation for this does not exist. Objectives. The authors present a case of numerical chromosomal abnormality. Method. Case report. Results. We present the case of a 5-month-old infant who presented at birth a plurimalformative syndrome characterized by craniofacial

dysmorphism, dermatoglyphic anomalies, bone abnormalities. Cytogenetic analysis revealed mosaicism 47, XY (8) / 47, XY (21). Conclusions. Very rare anomaly. Mosaicism trisomy 21 with trisomy 8 is rarely reported in the literature, only one case is reported.

PS13.29
Characterization of type-1 NF1 deletions

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Neurofibromatosis type 1 (NF1) is caused by mutations in the NF1 gene. In 5% of all NF1 patients large deletions encompassing the entire NF1 gene and its flanking regions are detected. The majority of these deletions span 1.4-Mb (type-1 NF1 deletions) and are characterized by breakpoints located within low-copy-repeats termed NF1-REPa and NF1-REPc. Previous studies have suggested that most type-1 NF1 deletion breakpoints cluster within hotspots of nonallelic homologous recombination (NAHR) termed PRS1 and PRS2, but a methodical analysis of breakpoint position in a large number of type-1 NF1 deletions has not so far been performed. The aim of our present study is to determine the precise breakpoint positions in 67 patients with type-1 NF1 deletions initially identified by MLPA in order to identify their breakpoints (sites of strand exchange) at the highest possible resolution. To do so, we performed SNP genotyping of paralog-specific PCR-products as well sequence analysis of breakpoint-spanning PCR-products. Our analysis indicates that 52 of the 67 type-1 NF1 deletions investigated harboured breakpoints within the 2-kb PRS2 hotspot. Only 12 deletions (18%) had breakpoints located within PRS1 whereas 3 deletions exhibited breakpoints that were located outwith PRS1 and PRS2. Our findings indicate that PRS2 is a very strong NAHR hotspot since it harbours 78% of all type-1 deletion breakpoints. The sequence analysis of the regions of strand exchange in all type-1 NF1 deletions promises to provide further information about recombination initiation sites and the processing of the recombination intermediates of type-1 NF1 deletions.

PM13.30
Nonsyndromic cleft lip and palate: identification of a causal element at 13q31

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Nonsyndromic cleft lip and palate (nsCLP) is one of the most common birth defects and has a multifactorial etiology. In a recent meta-analysis we identified a risk locus on chromosome 13q31, with the top-associated variant mapping to a non-coding region (~210 kb upstream of *SPRY2*). Here, we indented to follow-up this GWAS finding by (i) identifying the putative causal SNP, (ii) functionally annotating the top-associated region and (iii) understanding the biological relevance that explains the genetic association *in vivo*. Based on imputation results, we identified rs1854110 to be the functional candidate SNP ($P_{imputing}$=1.96×10^{-11}, relative risk=1.38 (95% confidence interval: 1.19-1.60). This marker was found to be located in a putative regulatory enhancer element relevant for craniofacial development. We cloned this element, together with a conserved region shown to be an enhancer region in neural crest cells, into a Tol2-vector system. First results revealed that zebrafish embryos injected with the enhancer element show GFP expression in cephalic regions. In order to determine the exact nature of GFP-positive cells, further experiments using immunostaining with antibodies against neural crest cell-specific markers will be conducted.
Our study provides first hints towards a specific regulatory element at an nsCLP-associated risk locus detected by GWAS. Further studies are currently ongoing to confirm and follow-up the initial findings, including a quantitative luciferase assay for the risk allele. Our studies will provide deeper insights into the biological mechanism underlying nsCLP association on chromosome 13q31.

PM13.32
Duplication of SHANK3 gene in a case of schizophrenia associated with autism

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The autistic spectrum is comprised of the Asperger syndrome, Heller syndrome, Rett syndrome, atypical autism, disorganized schizophrenia, as well as, although somewhere marginally placed, schizoid personality disorder and obsessive compulsive disorder.
We present a case of a 21 years old female with a psychopathological picture that consisted of: psychomotor agitation, a suicide attempt by defenestration, which was secondary to delusional ideation, auditory and visual hallucinations, anxiety, lack of insight, mixed insomnia. Our case, diagnosed with schizophrenia, also presents manifestations that are characteristic for autistic spectrum disorders. Based on her academic performances, our patient might have a superior than average intellect.
The result of the arrayCGH analysis showed genomic imbalances including several genes. From the list of deleted/duplicated genes, one that was previously reported in patients with psychotic disorders is the SHANK3 gene. ArrayCGH analysis for our case identified an 82.3 Kb duplication of chromosome 22q from 51.055.575-51.137.968 bp; this region encompassed the SHANK3. The patient's mother did not carry the duplication. The genetic analysis for father was not possible because he is deceased. Several papers have reported SHANK3 gene variants in autistic spectrum consisting of both copy number variants and point mutations.
The presence of three copies of the SHANK3 gene was reported in association with autistic spectrum disorders in other papers. These findings led to the idea that both haploinsufficiency, as well as an over-expression of the SHANK3 gene are associated with manifestations of autistic spectrum disorders and other neurobehavioral abnormalities.

PS13.33
Unbalanced translocation (X;13) as a cause of two diverse phenotypes in siblings due to duplication versus haploinsuficiency of SHOX gene and skewed inactivation pattern.

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We report a male patient with an unspecific facial stigmatization, developmental delay, microcephaly and a tall stature. His G - banded karyotype was normal. FISH of subtelomeric regions revealed deletion in subtelomeric part of chromosome 13q and a duplication in Xpter. These findings were due to a balanced translocation (X;13) present in proband's mother.
Array CGH revealed that the Xp duplication spans 3.5 Mb and contains 27genes from OMIM database including SHOX gene. The deletion on 13q spans 5 Mb and contains 19 genes from OMIM database.
We performed FISH examination of proband´s apparently healthy sister and revealed the deletion of SHOX gene region (Xp22.3) and a duplication of 13qter. Haploinsuficiency of SHOX gene resulted in previously undiagnosed Léri-Weill syndrome (short stature, mesomelia of upper limbs and Madelung's deformity of the wrists). Because of the skewed inactivation pattern with silencing of the derived X chromosome there was no impact of the partial trisomy 13q.

PM13.34
Chromosomal rearrangements in the 11p15 imprinted region: sixteen new 11p15.5 duplications with associated phenotypes and putative functional consequences

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The 11p15.5 imprinted region contains two clusters of genes which play an important role in prenatal and postnatal growth and which are controlled by two imprinting centers, ICR1 (Imprinting Control Region 1) and ICR2 (Imprinting Control Region 2).
Opposite genetic and epigenetic anomalies of this region result in two distinct syndromes with growth disturbance: Beckwith-Wiedemann syndrome (BWS, MIM #130650), characterized by overgrowth and Silver-Russell syndrome (SRS, MIM #180860), characterized by growth retardation.
Cytogenetic anomalies are rare in both syndromes and represent less than 3% of SRS or BWS. Large duplications encompassing the two imprinting centers have already been described as associated with SRS or BWS, depending on the parental origin of the duplication. However, smaller Copy Number Variants (CNV), encompassing totally or partially one or another of the imprinting centers have only been recently reported. These CNV are

sometimes difficult to interpret and require the help of clinical features and methylation profile.
We report here 16 new SRS or BWS patients with 11p15 microduplication encompassing either both domains ICR1 and ICR2, either one of them or only part of one of them, and discuss the possible functional consequences of the rearrangements after a review of the literature.

PS13.35
Mosaic patterns can be missed by SNP array because of absence of heterozygosity in the normal cells

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Introduction: SNP array allows the integration of genotype and copy number information, which permits the simultaneous detection of (low-level mosaic) copy number variations, consanguinity and UPD in cases of isodisomy. We report on a case where a mosaic pattern was missed by SNP array due to the absence of heterozygosity in the normal cells.
Case presentation: Array analysis of non-consanguineous parents was performed because the array result of their child showed a terminal deletion of at least 4.3 Mb in 6q27 (chr6: 166,863,544-qter). An Illumina Omni Express 850K was used for the trio analysis and the mother showed the same deletion. While the mother is healthy, the child presented with moderate developmental delay, autism and delay of motor skills. Distal 6q deletions have been described in literature and the patients' main characteristics are intellectual disability, hypotonia, seizures, brain anomalies and specific dysmorphic features. At least part of the clinical features of the child can be explained by this deletion. Because the mother has no phenotypic features we considered a mosaic pattern for the deletion. Therefore we performed FISH resulting in detection of the deletion in 49 of the 100 analyzed metaphases indicating that the mother is indeed a mosaic carrier for the deletion.
Conclusion: The reason we missed the mosaic pattern and only detected the deletion must have been caused by the absence of heterozygosity of the critical region. Therefore, we recommend FISH analysis in case a healthy carrier has a deletion to detect a possible mosaic pattern.

PM13.36
Non-sequential and multi-step splicing of the dystrophin transcript

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The long (2.2Mb) human dystrophin transcript (DMD) takes 16 hours to be transcribed and is co-transcriptional spliced. The presence of long introns (24 over 10kb long, 5 over 100kb long) poses challenges to the splicing machinery. Additionally, the heterogeneity in intron size suggests that intron removal not always takes place consecutively. Here, we explored the order of intron removal and potential multi-step splicing for the DMD transcripts in human skeletal muscle cell lines. A customized library of probes covering all exons and introns, has been generated capturing pre-mRNA and targeting DMD, followed by Hiseq sequencing. We developed a new pipeline (SplicePie) to analyze capture-pre-mRNA-sequencing data. Analysis showed that DMD introns can be removed non-sequentially generating exon blocks - joined blocks of exons flanked by unspliced introns. Exon blocks were detected by analysis of the coverage of paired-end reads and validated experimentally using PCR and Sanger sequencing. No correlation between intron length and speed of intron removal was observed. Computational analysis and experimental validation revealed that intron removal takes place in several steps for the majority of dystrophin introns. We found two mechanisms of multi-step intron removal in DMD- recursive and nested splicing. Non-sequential and multi-step splicing events were found throughout the DMD gene across three cell lines. We believe that our findings of non-sequential and multi-step splicing provide insight in the splicing mechanism and will be useful to optimize therapeutic strategies that interfere with the splicing process.

PS13.37
Non-mosaic structural chromosome rearrangements predisposing to larger mosaic ones: evidences for the existence of locus-specific constitutional chromosome instability

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The origins of somatic genome variations manifesting as structural chromosomal rearrangements remain largely unknown. However, it is generally accepted that susceptibility to structural chromosome aberrations can be produced by alterations to DNA sequences flanking the breakpoints or to rearranged chromosomal regions (i.e. CNV). Addressing genome variations in children with intellectual disability, autism, epilepsy and/or congenital malformations (n=200) by high-resolution CNV analysis (molecular karyotyping; resolution: 1 kbp or higher), we have surprisingly found that somatic structural chromosome abnormalities can co-occur with non-mosaic ones within the same loci. More precisely, we detected 8 cases (4%) (4 duplications and 4 deletions) demonstrating small non-mosaic structural chromosome rearrangements encompassed by larger mosaic ones. The duplications were mosaic dup3p26.3p26.1 (5.8 Mb) vs. regular dup3p26.1 (2.9 Mb); mosaic dup14q32.13q32.2 (4.5 Mb) vs. regular dup14q32.2 (1.8 Mb); mosaic dup17p13.1p11.2 (6.9 Mb) vs. regular dup17p12 (1.4 Mb); mosaic dup18p11.32p11.31 (5.3 Mb) vs. regular dup18p11.32p11.31 (4.8 Mb). The deletions were mosaic del5q35.1q35.3 (9.2 Mb) vs. regular del5q35.2q35.3 (2.3 Mb); mosaic del14q11.2 (2.2 Mb) vs. regular del14q11.2 (0.5 Mb); mosaic del15q13.1q14 (8.2 Mb) vs. regular del15q13.2q13.3 (2 Mb); mosaic delXp22.32p22.2 (4.7 Mb) vs. regular delXp22.31 (0.7 Mb). These findings allowed us to hypothesize small regular structural variations (CNV) to be able to produce locus-specific constitutional chromosome instability resulting in larger somatic rearrangements. Our speculations are also supported by previous communications reporting specific local genomic changes to underlie common microdeletions/microduplications at several of the aforementioned loci. Supported by Russian Scientific Fund (Grant #14-15-00411).

PM13.38
Utilising CRISPR/Cas9 genome editing to validate novel genes associated with telomere length in humans

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Introduction: Telomere length (TL) shortens with each round of cell division due to the inability of DNA polymerase to fully replicate the 3' end of the DNA. TL is therefore regarded as a marker of cellular age and has been proposed as a marker of biological ageing. Shorter mean leukocyte TL has been shown to be associated with several age-related diseases. GWA studies have identified seven loci that associate with telomere length in humans. Of these loci, five contain genes with known roles in telomere biology (TERC, TERT, NAF1, OBFC1, RTEL1) but the remaining two do not, therefore potentially identifying novel genes involved in telomere length determination in humans. In order to investigate this we have employed a CRISPR/Cas9 genome editing approach to explore the function of genes within one of these loci (ACYP2/TSPYL6) with respect to telomere length maintenance.
Materials and methods: We have used the CRISPR/Cas9 system to knockout ACYP2 and TSPYL6 as well as telomerase reverse transcriptase (TERT) as a positive control in human induced pluripotent stem cells. Lines have undergone multiple passages and TL is currently being measured by Q-FISH and qPCR.
Results: We have successfully created knock-out lines for ACYP2, TSPYL6 and TERT individually as well as ACYP2/TERT and TSPYL6/TERT double knock outs. We will present preliminary data on telomere length maintenance in these lines.
Conclusions: We have utilised the CRISPR/Cas9 genome editing system as a means of investigating the role of novel genes in the control of telomere length in humans.

PS13.39
Breakpoint analysis of the recurrent constitutional t(8;22)(q24.13;q11.21) translocation

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The t(8;22)(q24.13;q11.2) has been identified as one of several recurrent constitutional translocations mediated by palindromic AT-rich repeats (PATRRs). Although the breakage on 22q11 utilizes the same PATRR as that of the more prevalent constitutional t(11;22)(q23;q11.2), the breakpoint regi-

on on 8q24 has not been elucidated in detail since the analysis of palindromic sequence is technically challenging. In this study, the entire 8q24 breakpoint region has been resolved by next generation sequencing. Eight polymorphic alleles were identified and compared with the junction sequences of previous and two recently identified t(8;22) cases . All of the breakpoints were found to be within the PATRRs on chromosomes 8 and 22 (PATRR8 and PATRR22), but the locations were different among cases at the level of nucleotide resolution. The translocations were always found to arise on symmetric PATRR8 alleles with breakpoints at the center of symmetry. The translocation junction is often accompanied by symmetric deletions at the center of both PATRRs. Rejoining occurs with minimal homology between the translocation partners. Remarkably, comparison of der(8) to der(22) sequences shows identical breakpoint junctions between them, which likely represent products of two independent events on the basis of a classical model. Our data suggest the hypothesis that interactions between the two PATRRs prior to the translocation event might trigger illegitimate recombination resulting in the recurrent palindrome-mediated translocation.
These studies were supported by a grant-in-aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

PM13.40
Down syndrome caused by mirror duplication of chromosome 21 : conventional cytogenetic, FISH and array CGH

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Down syndrome (DS) is a well-known syndrome; the frequency is about 1 in 2000 live births. The most common form (95%) is due to an extra copy of chromosome 21 in all cells, in 2% of the cases the trisomy is a mosaism, and 2% present with robertsonian translocation. In the last 1%, it is due to a structural abnormality in the 21q22 region.
However a rare variant is the duplication of the chromosome 21 with mirror duplication (reverse tandem). It is characterized by partial duplication and partial deletion of chromosome 21.
Here, we report the case of a child with typical DS features. At birth, we performed standard karyotype which showed that she carried mirror duplication of the chromosome 21.
An array comparative genomic hybridization was performed to define exactly the triplication and to highlight a possible monosomy. It showed a duplication of 29,9 Mb of the region 21q11.2q22.3 and a deletion of 2,8Mb of the region 21q22.3. The couple has been through 3 miscarriages.
After the birth of their third child, the analyses were completed in order to highlight a paracentric inversion or other chromosomal rearrangement in chromosome 21 in the parents' karyotype.
After such a karyotype for the children and the antecedent of miscarriages for the couple, it is necessary to go further in the analysis, and to look for chromosomal rearrangement in the karyotypes of the parents. This can explain that association of duplication deletion, and adjust the genetic counselling.
Furthermore the description of those trisomies 21 with duplication deletion helps to refine a genotype-phenotype correlation.

PS13.41
X-linked ichthyosis in a patient with a novel nonsense mutation in the STS gene.

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X-linked recessive ichthyosis (XLI) is a genodermatosis due to steroid sulfatase deficiency. XLI is characterized by dark, adhesive, polygonal and regular scales and presents a frequency of 1 in 2000-6000 males. Ninety percent of XLI patients have deletions of the entire STS gene and flanking sequences; currently, 14 point mutations and seven partial deletions have been reported worldwide. The aim of the present study is to describe the STS gene in a patient with X-linked ichthyosis and a mild phenotype. XLI diagnosis was confirmed through steroid sulfatase assay in leukocytes. Genomic DNA was extracted through conventional methods. Exons 1-10 of the STS gene were analyzed by polymerase chain reaction and DNA sequencing analysis. XLI proband had undetectable levels of STS activity. The DNA sequence analysis showed a novel nonsense mutation on 3 exon of the STS gene producing a stop codon. This data enriches the mutational spectrum of the STS gene in XLI patients and remarks the variability in the phenotypic spectrum independently of the molecular defect.

PS14.001
Rapid and portable Lab-on-chip genotyping for Clopidogrel metabolism at the point-of-care

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Background. Effective platelet inhibition has become a cornerstone in the management of patients with acute coronary syndromes (ACS). The current standard of care includes dual antiplatelet therapy with aspirin, and one of the three currently available ADP P2Y12 inhibitors: clopidogrel, prasugrel and ticagrelor. We used a novel point-of care lab-on-chip tool in order to genotype patients experiencing ACS to identify the carriers of the ABCB1 3435, CYP2C19*2 and CYPC2C19*17 alleles and so assessing a pharmacological approach incorporating these genetic variants.
Methods and Results. Between October 2013 and January 2015, 800 patients were enrolled into a two-armed, single blind, randomized controlled trial assessing the clinical usefulness of adding pharmacogenomic data on top of clinical variables when choosing dual antiplatelet treatment. In the pharmacogenomic arm, genotyping was done at the patients' point-of-care by means of a newly developed portable real-time PCR system (Q3) by scoring the CYP2C19*2, CYP2C19*17 and ABCB1 3435 alleles in a turnaround time of 70 min from DNA extraction to final genotype calls. When compared to other gold-standard conventional laboratory genotyping techniques, the newly developed system showed 100% concordance.
Conclusions. The Q3 system proved to be as reliable as the current available techniques. Genotyping in the ACS setting should not be delegated to centralised clinical laboratories for matter of time however the genotyping at the patients' bedside represents the opportunity to realise large, randomized trials in order to assess the clinical improvement of adding genotype data (gathered bed-side) to the classical clinical variables for the outcomes of patients with ACS.

PM14.002
Fully automated Ion AmpliSeq™ library preparation using the Ion Chef System

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As next generation sequencing evolves, the ability to create reproducible, high quality libraries becomes increasingly important. The Ion AmpliSeq™ method has proven useful to reliably generate quality libraries from as little as 10 ng of nucleic acid. We developed the Ion Chef system to offer an automated solution for templating and sequencing chip preparation. Here we demonstrate the marriage of Ion AmpliSeq™ reagents with the Ion Chef system to create a fully automated library preparation solution. This system includes a new set of consumables and software to enable DNA to loaded chip in less than 24 hours with only two touch points. Eight Ion AmpliSeq libraries can be prepared in less than 7 hours with less than 15 minutes of hands on time. Using an 8000 amplicon targeted cancer panel we observe >95% uniformity for all 8 samples, which compares favorably to manually prepared libraries. Additionally, using Equalizer™ normalization we observe highly reproducible barcode balance with less than 10% CV between libraries. We have seen similar high performance with a variety of panels ranging from 50 to 25,000 amplicons from high quality genomic DNA as well as FFPE DNA. With the pushbutton automation of Ion AmpliSeq™ library preparation, we boast the first NGS system with a fully automated workflow from sample to answer.

PS14.003
Next generation sequencing-based molecular diagnostics for Autosomal Recessive Polycystic Kidney Disease

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Introduction: Autosomal recessive polycystic kidney disease is characterized by cysts development in the collecting ducts often associated with hepatic involvement and it is caused by mutations in *PKHD1* gene. Due to the large size of this gene the conventional mutation screening is time-consuming and expensive, so that a molecular diagnosis is often missed.
Materials and Methods: A NGS method for screening of *PKHD1* gene based on Ion Torrent platform was developed and validated in this study. 26 subjects were submitted to our NGS protocol, 14 with known mutations in *PKHD1* gene and 12 without a molecular characterization.
Results: Our NGS method allowed to detect all the mutations previously identified by conventional sequencing. Additional mutations were also identified, providing more information about the carrier status or completing the molecular characterization in probands. All additional or newly detected variants were confirmed by Sanger sequencing. Our NGS approach allowed to identify the mutations also in all the not yet tested subjects. During our analysis 17 previously undescribed variants were detected, contributing to extend the spectrum of known mutations in *PKHD1* gene.
Conclusions: This method resulted accurate, robust and more sensitive than the conventional sequencing, with a considerable reduction of work times and costs. Although it will be necessary to test more patients, our study is a proof of concept showing the feasibility of our NGS protocol for diagnostic purpose, improving and accelerating molecular diagnosis and genetic counseling for families.

PM14.004

Revisiting previously classified Copy Number Variants - Is it worthy for management of clinical strategy?

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Microarray-based comparative genomic hybridization (array-CGH) allows the possibility to screen the whole genome at once and with high resolution, allowing the detection of Copy Number Variants (CNVs). Taking into account the existence of known syndromes, relevant genes for the phenotype and CNVs in healthy population, we have proposed the classification of CNVs into different classes: Class I are deletions/duplications in regions associated with a syndrome; Class II are deletions/duplications not reported in normal subjects and involving known coding genes; Class III are /duplications reported in low frequency in normal subjects or not involving genes; Class IV are deletions/duplications reported in healthy subjects -common variants.
We have revisited 250 cases with intellectual disability and/or Autism spectrum disorders, analyzed by oligonucleotide array-CGH (Agilent 4x180K platform) from 2011 and 2012 in order to evaluate if the observed CNVs would be reclassified according to the update of databases.
In~70% of the reviewed cases there were no changes in the CNVs' classification. In the other ~30% of cases: 1 classification changed from Class II to I, 10 from II to IIIA, 5 from II to IV, 17 from IIIA to IV, 3 from IIIB to IIIA, 45 from IIIB to IV. About 95% of the classification changes made reduced the probability of the imbalance being responsible for the phenotype.
Although progress and knowledge have improved the content of databases, the findings of this revision have not changed the clinical strategy neither the management of patients. We can conclude that our CNV classification system is robust.

PS14.005

High-resolution array CGH analysis of patient samples in the Deciphering Developmental Disorders study.

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One of the aims of the DDD project was to perform array CGH analysis on a large cohort of child patient samples using a 2x 1 million feature Agilent microarray. A high-throughput pipeline was established to carry out this analysis.
Patient DNA was derived from blood or extracted in house from saliva. Saliva samples were collected using Oragene kits and DNA was extracted using a QiaSymphony robot. For array CGH analysis samples were fluorescently labeled using an Agilent commercial kit and a BRAVO robot. Samples are combined with a reference DNA (pooled DNA from 500 male blood donors) and hybridized for 3 nights in batches of 24. The arrays were then washed in batches of 8 using a Little Dipper wash station. Arrays were scanned using an Agilent G2565CA scanner, and the data extracted using Feature Extraction 10.5. Arrays were handled in a humidity, temperature and ozone controlled environment. 95 patient samples were processed within 10 days. An in house LIMS tracked samples through the pipeline.
We have analysed over 1000 normal control samples, generating a control data set to aid interpretation of patient data. We have now processed ~7,700 DDD patient samples. Patient DNA samples typically produce a median DLRs value of 0.17. Copy number changes are detected using an in house analysis pipeline and the inheritance status evaluated by comparison with exome CNV data. Variants identified were assessed in attempt to identify the cause of the child's developmental disorder.
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PM14.006

An efficient NGS tool for cascade genetic screening of Familial Hypercholesterolaemia

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Introduction Familial hypercholesterolemia (FH) is a common autosomal-dominant disorder caused by mutations in known genes (LDLR, APOB or PCSK9). In addition, APOE may be considered as the 4th locus of autosomal-dominant hypercholesterolemia (ADH) and the main modifier-gene of FH and cardiovascular risk. Cascade screening of FH using genetic testing for ADH has been suggested by international guidelines but a proportion of patients may have a polygenic cause which could compromise the efficiency of cascade testing. Recently Talmud et al. described a polygenic score that helps identifying the polygenic contribution in FH. We have developed a NGS tool combining the detection of monogenic as well as polygenic forms of FH.
Materials and Methods Complete coding regions of LDLR, PCSK9 and APOE, and a specific region (c.10200 to c.11100) of APOB are amplified, generating 65 amplicons in 4 multiplex PCR reactions (ADHMASTR kit developed in collaboration with Multiplicom). Ten control fragments are added for the detection of Copy Number Variations (CNV). A fifth plex was added corresponding to fragments containing the 12 SNPs used for the polygenic score.
Results Multiplexed PCR products of 96 patients are sequenced in each MiSeq run (V3 chemistry). Bioinformatic analysis allowed us to detect point mutations in the 4 genes analyzed as well as CNV. In FH patients without identified mutation, the polygenic score was calculated to classify them as of polygenic origin.
Conclusions We have developed an efficient and cost-effective NGS tool allowing the detection of monogenic and polygenic forms of FH. This kit is well adapted to high-throughput cascade screening of ADH.

PS14.007

Diagnostic value of methylation analysis by pyrosequencing in Beckwith-Wiedemann and Silver-Russell syndromes

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Beckwith-Wiedemann syndrome (BWS) and Silver-Russell syndrome (SRS) are characterized by opposite clinical findings. Main features of BWS are pre-postnatal macrosomia, macrogglossia, ear crease and/or pits, visceromegaly, abdominal wall defects, hemihyperplasia and increased risk for embryonic tumors. In contrast to these findings, SRS is characterized by pre-post natal growth retardation.
BWS and SRS are associated with epigenetic alterations of chromosome 11p15.5. Cytogenetical methods can identify alterations of chromosome 11p15.5 in fewer than 1% BWS patients. The rate to detect epigenetic alterations of chromosome 11p15.5 in BWS patients by molecular methods is 75-80%. 50% of SRS patients can be diagnosed genetically by demonstrating epigenetic alterations of chromosome 11 and 7. Methylation-spesific Multiplex Ligation-Dependent Probe Amplification (MS-MLPA) is being used efficiently in a few laboratories to detect epigenetic alterations. In recent studies, methylation specific pyrosequencing (MS-pyrosequencing) was performed to confirm clinical diagnosis of BWS and SRS patients and

it was suggested to be more powerful than MS-MLPA. The purpose of our study is to demonstrate the diagnostic value of MS-pyrosequencing in our BWS and SRS patients.

PM14.008

Validation of the Beta-Thal Modifier StripAssay: A novel test predicting disease severity of beta-thalassemia

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Background: The clinical phenotype of patients with beta-hemoglobinopathies is extremely heterogenous, ranging from nearly asymptomatic forms of thalassemia intermedia to severe transfusion dependent thalassemia major. The wide phenotypical variability is associated with the type of beta-globin mutation, the coinheritance of alpha-thalassemia and the ability for persistent production of fetal haemoglobin (HbF) in adult life. Three major quantitative trait loci, accounting for 20-50% of HbF variation, have been identified by now. Single nucleotide polymorphisms in the gamma-globin gene promoter (*HBG2*), in the *BCL11A* gene and *HBS1L-MYB* intergenic region contribute to the calculation of the Thalassemia Severity Score (TSS), a free web-based tool (http://tss.unica.it/home/#.VNNSCS7CT2c) for the prediction of clinical severity.
Methods: We developed a teststrip-based reverse-hybridisation assay for the simultaneous detection of polymorphisms in quantitative HbF loci: *Xmn*I (g.-158 C>T, rs7482144) in *HBG2*, rs1427407 and rs10189857 in *BCL11A*, and rs28384513 and rs9399137 in the *HBS1L-MYB* region. A total of 75 pretyped samples were retested in three different French thalassemia centers for assay validation.
Results: StripAssay results were 100% concordant with genotypes previously obtained by reference methods in the three participating laboratories. The Beta-Thal Modifier StripAssay quickly and correctly identifies disease modifying alleles and thus supports the use of TSS for the prediction of patients likely to display less severe phenotypes.
Conclusions: Testing for genetic modifiers allows a more specific and effective treatment and may also support clinical decisions regarding the onset of transfusion therapy in beta-thalassemia patients. Furthermore, the knowledge about prognostic markers could find an implication in the future, in genetic counselling and prenatal diagnosis.

PS14.009

Bisulfite conversion of DNA: performance comparison of different kits and methylation quantitation of epigenetic biomarkers used in Non-invasive Prenatal Testing

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Epigenetic alterations, including DNA methylation, play an important role in the regulation of gene expression. Several methods exist for evaluating DNA methylation but bisulfite sequencing is the gold standard. The challenge of the method is that the desired outcome (conversion of unmethylated cytosines) positively correlates with the undesired side effects (DNA degradation and inappropriate conversion), thus several commercial kits try to adjust a balance between the two. Here, we compared the performance of four bisulfite conversion kits [Premium Bisulfite kit (Diagenode), EpiTect Bisulfite kit (Qiagen), MethylEdge™ Bisulfite Conversion System (Promega) and BisulFlash DNA Modification kit (Epigentek)] regarding conversion efficiency, DNA degradation and conversion specificity.

Performance was tested by combining fully methylated and unmethylated λ-DNA controls in a series of spikes and we assessed them by Sanger sequencing (0%, 25%, 50% and 100% methylated) and Next-Generation Sequencing (0%, 3%, 5%, 7%, 10%, 25%, 50% and 100% methylated).

We also studied the methylation status of two of our previously published differentially methylated regions (DMRs) at base resolution by using spikes of CVS in whole blood. The kits showed different but comparable results regarding DNA degradation, conversion efficiency and conversion specificity. However, the best performance was observed with the MethylEdge™ Bisulfite Conversion System (Promega) followed by the Premium Bisulfite kit (Diagenode). The DMRs were confirmed to be hypermethylated in the CVS and hypomethylated in whole blood. Finally, we showed that bisulfite amplicon sequencing is a suitable approach for methylation analysis of targeted regions.

PM14.010

Molecular inversion probe based BRCA1/2 re-sequencing in a clinical setting

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Mutations in BRCA1 and BRCA2 confer high risks of hereditary breast and ovarian cancer. Over the years capillary Sanger sequencing and MLPA were used to detect causal variants in breast cancer families. Next generation sequencing technologies allowed implementing new strategies thereby reducing costs without losing sensitivity. Today sequencing costs of targeted NGS-based sequencing strategies are largely determined by enrichment procedures. Molecular inversion probes (MIPs) have shown to be a cost-effective enrichment used in multiplex, particularly if used in large cohorts. Here we present a workflow of re-sequencing BRCA1 and BRCA2 using single molecule molecular inversion probes (smMIP) in combination with NexSeq500 sequencing. The strategy involves the analysis of all coding exons and their flanking intronic sequences. We designed 402 overlapping smMIPs on both strands avoiding known SNPs in probe binding sites. Every base was targeted by at least two independent smMIPs. This yielded 100% coverage of all targeted bases. Next all BRCA1 and BRCA2 positive cases in our laboratory from 01/2010 till 07/2014 were sequenced for both genes, these were 103 BRCA1 and 71 BRCA2 disease causing mutations. All disease causing mutations and all known polymorphisms in both genes were detected, including one SNP that was missed using capillary sequencing due to allelic dropout. No false positives were reported.
In conclusion, targeted BRCA1 and BRCA2 re-sequencing using smMIPs shows high sensitivity and specificity and can replace sequencing workflows in a clinical setting. smMIPs allow low enrichment costs per sample and highly scalable workflows.
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PS14.011

Next-generation sequencing of the BRCA1 and BRCA2 genes for the genetic diagnostics of hereditary breast and/or ovarian cancer

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Genetic testing for hereditary breast and/or ovarian cancer (HBOC) mostly relies on laborious molecular tools that use Sanger sequencing to scan for mutations in the BRCA1 and BRCA2 genes. We have explored a more efficient strategy based on next-generation sequencing (NGS) of the BRCA1 and BRCA2 genes in 210 (HBOC) patients. We first validated this approach in a cohort of 115 samples with previously known BRCA1 and BRCA2 mutations and polymorphisms. Genomic DNA was amplified using the Ion AmpliSeq™ BRCA1 and BRCA2 panel. The DNA Libraries were pooled, barcoded and sequenced using an Ion Torrent PGM sequencer. The combination of robust bioinformatics tools allowed us to detect all previously known pathogenic mutations and polymorphisms in the 115 samples, without detecting spurious pathogenic calls. The assay achieved a sensitivity of 100% (95% CI: 99.71% to 100%), with a specificity of detecting non-variant sites from the reference sequence of 99.99% (95% CI: 99.99% to 100%), a positive predictive value of 91.17% (95% CI: 89.72% to 92.62%), and a negative predictive value of 100% (95% CI: 100% to 100%). We then used the same assay in a discovery cohort of 95 uncharacterized HBOC patients for BRCA1 and BRCA2. In addition, we describe the allelic frequencies across 210 HBOC patients of 74 unique definitely and likely pathogenic, and uncertain BRCA1 and BRCA2 variants, some of them not previously annotated in the public databases. Targeted NGS is ready to substitute classical molecular methods to perform genetic testing on the BRCA1 and BRCA2 genes, and provides a greater opportunity for more comprehensive testing for at-risk patients.

PM14.012

A comparative study of *BRCA1*/2 mutation screening methods in use in European clinical diagnostics laboratories.

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BRCA1/2 mutation screening is offered by a large number of clinical laboratories in Europe. Practice is changing rapidly due to increased demand for testing, the advent of treatment focussed genetic testing and the rapid uptake of next-generation sequencing technologies.
Following a survey of current *BRCA1/2* mutation screening methods circulated to ~1500 members of the European Molecular Genetics Quality Network, 20 laboratories were selected to participate covering a representative range of standard (Sanger sequencing and mutation scanning) and NGS methodologies including different platforms and library preparation methods. The selected laboratories reported a significant clinical caseload per annum.
Eight cell line DNA samples with a range of *BRCA1/2* mutation types and 2 samples with no mutations were distributed to participating laboratories. Laboratories were given two months to complete their analyses and asked to report significant findings (would be include in a clinical report) and all differences from a reference sequence.
There was no significant trend identified with respect to the genotyping accuracy of the different methodologies. Seventeen (85%) study laboratories identified all clinically significant *BRCA1/2* variants- no false-positive mutation calls were made. Four genotyping errors (false-negatives) were made by three laboratories: A comparison of the variant calls across samples showed 84% concordance of the different coding variants detected across all samples. For these variants, there was a mean score of 99% concordance of variant calling between the laboratories.
All methods used by experienced clinical laboratories performed well on a challenging range of *BRCA1/2* mutations. The errors identified are likely due to problems with downstream analyses processes rather than the wet-lab methodology.

PS14.013

Sensitive detection of whole exon deletion/duplications using the TruSight Cancer panel

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Targeted next generation sequencing (NGS) panels are increasingly being used in clinical genomics to increase volume, throughput and affordability of gene testing. Identifying whole exon deletions/duplications in NGS panels that only target coding regions has proved challenging, particularly for single exon variants. Many labs currently use a separate method for their detection, adding to the test cost and turnaround.
We are using the Illumina TruSight Cancer Panel (TSCP) to test for mutations in cancer predisposition genes in a clinical testing laboratory, TGLclinical. We process 48 samples at a time, and sequence using a HiSeq2500 (2x48), generating median coverage of 500X across the panel.
After evaluation of multiple tools we selected and optimised ExomeDepth for use with exon targeted NGS panels. We evaluated its performance using 96 samples with independently validated data, including 36 samples with large variants, primarily in BRCA1 or BRCA2. The optimised ExomeDepth achieved 100% sensitivity for BRCA1 and BRCA2 large variant detection, identifying all 21 mutations including 9 single exon deletion/duplications, while retaining a low false discovery rate of 0.09. We performed extensive simulations to confirm a high sensitivity for detection of single exon variants under a range of experimental parameters, and to establish robust criteria for a negative result. Finally, we implemented ExomeDepth in a clinical pipeline in 2,064 samples with validation by MLPA identifying 16 single exon deletion/duplications whilst maintaining a low false positive rate.
This work was undertaken by the MCG programme (www.mcgprogramme.com) funded by the Wellcome Trust Grant 098518/Z/12/Z.

PM14.014

Multi-gene panel testing in hereditary cancer: a diagnostic service review and recent developments

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The rapid expansion of NGS within diagnostic laboratories has led to the introduction of multi-gene services offering comprehensive mutation screening for complex disease. Services based on simultaneous investigation of large gene panels result in faster, more cost-effective tests. In 2014 we introduced an 82 gene hereditary cancer panel, based on local clinical demand. The gene list is fully customisable; therefore the panel is ideally suited for patients with complex personal and/or family histories of cancer.
Samples are tested using Agilent SureSelect chemistry and Illumina sequencing. To date, we have tested over 250 patients, achieving 100% coverage at 50x and a diagnostic yield (likely pathogenic and pathogenic variants) of 12%. As clinicians typically opt for a relatively small phenotype-focused subset of genes, the discovery of variants of uncertain clinical significance is minimised (13% reports). In the event of a negative result, additional genes may be analysed if appropriate without the need to perform further laboratory work.
A recent major development in our service is the adaptation of the NGS pipeline for the detection of large duplications and deletions using comparative depth of coverage analysis. Validation studies confirmed 325 individual gene results, including 38 dosage abnormalities. This method enables comprehensive dosage analysis on a larger scale and at a lower cost compared to using a stand-alone method e.g. MLPA.
As laboratory and analysis pipelines become increasingly automated, we aim to meet a turnaround time of 40 working days for all cancer referrals and anticipate a significantly reduced test cost, ultimately leading to improved patient care pathways.

PS14.015

Evaluation of NRF2 Gene Expression in CD133+ Stem Cells Derived from Umbilical Cord Blood of New Born Minor Beta Thalassemia Treated with Thalidomide and Sodium Butyrate

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Efficient induction of fetal hemoglobin (HbF) considers as an important and proper therapeutic approach in patients with beta thalassemia and sickle cell anemia.
Thalidomide and sodium butyrate are both considered as active HbF inducer medication with p38 MAPK activation potential for gamma globin gene induction. NRF2 as key molecule of p38MAPK signaling pathway upregulates following stress oxidation status and cause over-expression of gamma globin gene.Studying the importance of this molecule in the induction of HbF by thalidomide and sodium butyrate can cause more precise underestanding about molecular mechanisms interfering in HbF induction.
Therefore, in this study the effect of thalidomide and sodium butyrate on erythroid progenitors derived form CD133+ cells with minor beta thalassemia mutation was evaluated in invitro. Flowcytometry analysis shows about 96% purity of extracted CD133+ cells. Afterwards, this cells were cultured in erythroid differentiation medium.
In day 6 drug treatment was done and erythroid progenitors were analysed after 12 day of differentiation. Real-time PCR analysis showed significant increased expression of the NRF2 transcript in cell culture groups containing thalidomide and sodium butyrate as compared to control. Moreover, Real-time PCR analysis did not show significant increasing the expression of the NFE2 transcript in cell culture groups containing thalidomide and sodium butyrate as compared to control ($P<0.05$). With regard to the specific role of both drugs given in the induction of fetal hemoglobin expression, it seems that regulation of NRF2 can play an important role in the expression of gammaglobin gene.

PM14.016

Benefits from applying an extended CFTR screening protocol to a heterogeneous population

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Introduction: Cystic fibrosis (CF) (MIM#219700) is the second most common autosomal recessive disorder in Greece after thalassemias. The carrier frequency is estimated approximately at 3-4% of general population with an incidence of about 1 in 2500-3500 live births. The frequency and distribution of mutations show clear ethnic and geographical distribution and Greece, due to its geographic position, presents high mutational heterogeneity.
Materials and methods: The extended screening protocol included screening of the whole coding sequence, intron-exon boundaries, as well as screening for abnormal copy numbers (deletions and duplications) using MLPA® (SALSA MLPA P091 CFTR probemix). Screening was performed by High Resolution Melting Analysis (HRM) using LightScanner® (Idaho Technologies, Utah, USA) and all positive findings were confirmed by bi-directional Sanger

sequencing.
Results: This approach was applied to 94 patients selected due to having one or both undetermined causative mutations and we were able to identify both causative mutations in 59 of them. The protocol was also applied to 2300 individuals from risk groups (partners of CF carriers/patients, couples presenting with echogenic bowel at the second trimester of pregnancy, males with CBAVD) and 50 carriers were identified, that with other screening protocols would have been missed.
Conclusions: The extended protocol described herein proves to be necessary when screening an extremely heterogeneous population such as the Greek population. The necessity for identifying both causative mutations in a patient is especially important nowadays that innovative therapeutic agents available aim at specific genotypes.

PS14.017

Rapid detection method for the 4 most common CHEK2 mutations based on melting profile analysis

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CHEK2 is a suppressor gene, encoding a protein kinase, involved in the regulation and control of the cell cycle. Changes in the structure of CHEK2 affecting the functionality of the expression product are associated with an increased cancer risk in various organs. The elevated risk, in a significant percentage of cases, is determined by the presence of 4 most common mutations in the gene CHEK2, including three point mutations c.470T> C, IVS2 + 1G> A, c.1100delC, and one large rearrangement del5395. The substantial role of these variants in increasing the risk of various cancers leads to the need of development of methods for their detection.
Here we present a rapid and effective method for the detection of 4 most common CHEK2 gene mutations based on high-resolution melting (HRM) analysis, and its modification (C-HRM) enabling simultaneous detection of CNVs. The analysis is performed in two multiplex PCR reactions followed by melting analysis, without any additional reagents or handling beside that used in a standard HRM.
Validation of the method was conducted on a group of 103 patients with diagnosed breast cancer, a group of 176 members from families with cancer cases in organs associated with the CHEK2 gene mutations, and a control group consisting of 100 unrelated, healthy individuals from polish population.
The developed methodology enables to improve the genetic diagnostics of patients. Moreover, it reduces the cost of such analysis, and facilitates their implementation. Therefore, it will be possible to cover a larger group of patients, and at the same time, increase the efficiency of identification of people at risk.

PM14.018

Sensitive multiplex diagnostics of fusion genes in childhood leukemia using biochip

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Chromosomal rearrangements and fusion genes play key role in leukemogenesis and contribute to different leukemic subtypes. It is practical and helpful to detect the fusion genes in diagnostics of leukemia using inexpensive high-throughput methods. Standard reverse transcription polymerase chain reaction (RT-PCR) approach to detect the fusion transcripts is effective, but time-, labor- and patient material consuming. To simplify and accelarate parallel analysis of multiple targets, we established a method whish combined multiplex RT-PCR and microarray (biochip). The system allows detecting 22 most clinically important chromosomal rearrangements in acute leukemia generating more than 70 fusion gene variants (ETV6-RUNX1; BCR-ABL1 p190 and p210; MLL-AFF1; PML-RARA; RUNX1-RUNX1T1; TCF3-PBX1; MLL-MLLT3; CBFB-MYH11; MLL-MLLT10; MLL-MLLT1; SIL-TAL1; MLL-MLLT4; NPM1-ALK; TCF3-HLF; MLL-ELL; MLL-EPS15; PICALM-MLLT10; MLL-MLLT11; DEK-NUP214; RBM15-MKL1; FUS-ERG). Reverse transcription was performed using a set of specific primers and was followed by PCR with primers containing gene-specific sequences and universal adapters. The target was labeled during PCR by incorporation of fluorescent triphosphate analogs. The fluorescently labelled PCR products were hybridized with a biochip containing immobilized probes. To increase sensitivity of the approach new near-infrared labels (Cy7) were tested. The method allows detecting 1 leukemic blast in 10000 normal cells. Using the approach 100 clinical samples were screened for the presence of translocations. The detected fusion genes were validated with RT-PCR. Our data suggested that the RT-PCR-biochip approach could screen 22 fusion genes with high accuracy and sensitivity. The work is supported by Federal Target Program of Ministry of Education and Science of Russia (grant №14.604.21.0117, RFMEFI60414X0012).

PM14.020

Efficient diagnostic routing using clinical exome sequencing results in a high diagnostic yield of 28 %, limiting the need for whole genome sequencing.

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Clinical exome sequencing (CES) can provide a molecular diagnosis in families with an unexplained phenotype. When using this technique in a diagnostic setting it is important to follow a well documented diagnostic routing. Here we present an effective routing and the results of the first 129 families. Furthermore, we compare the diagnostic yield of our exome pipeline with the yield of Whole Genome Sequencing (WGS).
In a multidisciplinary team, candidate families are discussed and selected. The diagnostic routing prior to WES is defined: exclusion of other genes in differential diagnosis and deletions/duplications (array), revealing the regions of homozygosity (SNP array), and determination of the primary filtering strategy according to the expected inheritance model. Prioritization of variants is performed with Cartagenia Bench Lab NGS using validated variant filtering trees (de novo, recessive, X-linked, multiple affected AD/AR).
The diagnostic WES procedure was evaluated for the first 129 families. A conclusive diagnosis was made in 36/129 patients (28%), including intellectual disability (ID, n=90, yield 24%) and phenotypes without ID (n=39, yield 39%). Nine incidental findings were detected, two clinical diagnoses and seven carrierships.
Furthermore, we performed an in silico analysis of how well our strategy would have performed compared to WGS, by determining the number of mutations that we would have missed based on a large WGS study (Gilissen, Nature 2014). Our strategy would have identified 17/21 mutations, at much lower costs.
In conclusion, we have implemented an efficient strategy for CES. The diagnostic yield is high (28%), and this yield is close to that of WGS.

PM14.022

Establishing a clinical genetic testing laboratory to rapidly implement research advancements into clinical practice

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TGLclinical laboratory was established in Oct 2012 as a clinical testing laboratory operating to ISO 151589:2012 medical laboratory standards. TGLclinical is embedded within a long established research laboratory and clinical unit and was formed with the specific objective of translating sequencing technologies and analytical pipelines developed through research into immediate benefit for clinical services. We outline the timeline and processes involved in achieving this, including the pathway to achieving accreditation to ISO 15189 standards.
TGLclinical is now delivering testing for cancer predisposition genes to the Royal Marsden Hospital using next-generation sequencing with the Illumina TruSight Cancer Panel. This has increased throughput at greatly reduced costs and turn-around times. TGLclinical interpretation services are fully integrated with local clinical and research expertise and provide a standard of clinical interpretation that can be used effectively by both oncology clinicians during the routine care of patients with cancer and by genetic clinicians in standard genetic services.
The establishment of TGLclinical has enabled significant changes and improvements over the past 3 years to the genetic services available at the Royal Marsden Hospital. For *BRCA1* and *BRCA2* gene tests as an example, the charge per test has reduced by ~75%, the number of patients being tested has increased 3.5 fold and the average turn-around time for results has reduced from 8 weeks to less than 4 weeks. The establishment of TGLclinical was made possible through start-up funding from The Royal Marsden Cancer Charity.

PS14.023

Improving CNV detection from next generation exome sequence data (multiple algorithms and dynamic segment ranking)

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The Deciphering Developmental Disorders (DDD) is a project at the Wellcome Trust Sanger Institute focussing on identifying rare genetic variants in a cohort of approximately 12,000 patients with undiagnosed developmental disorders. Next Generation Exome sequencing and array comparative genomic hybridization (aCGH) are used to scan patient genomes for a wide range of genomic variation, including copy number variation (CNV).
Here we introduce a pipeline to perform CNV detection on large numbers of Next Generation Exome Sequencing datasets. Within this pipeline the following detection algorithms have been implemented: ADM3, CANOES, CoNIFER, CoNVex, ExomeDepth, GADA and XHMM. A new method has been developed for the combining and ranking of CNV detections, called: split segment ranking. This is a dynamic ranking system that converts confidence scores from existing algorithms into a new comparable ranked score.
We show that by combining the CNV detections of multiple algorithms we can improve call specificity, while retaining a solid number of detections. Using 500 samples from the DDD project we assessed the CNV calling rates across all algorithms in terms of sensitivity and specificity. By using the proportion of common CNV detections made as an estimate for specificity we show that by using multiple algorithms we achieved a common CNV detection rate of approximately 90% which is in line with previous studies into rare CNV. However, by implementing the split segment ranking method we can potentially achieve a similar specificity while retaining more CNV detections overall.

PM14.024

External assessment of competency to authorise diagnostic genetic reports

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The ISO 15189 standard requires laboratories to train and continually competency assess all levels of staff for a range of activities to ensure a high standard of testing is delivered. This includes demonstrating individual proficiency of staff authorising genetic test results. Laboratories often find it difficult to assess and provide evidence of the expertise of the process of report authorisation. Due to participant demand the UK National External Quality Assessment Service (UK NEQAS) for Molecular Genetics delivered a pilot external quality assessment (EQA) during 2014 to assess if EQA could help evidence this competency.
Twenty eight genetic laboratories participated in this pilot run and were supplied with three clinical case scenarios for patients referred for cystic fibrosis molecular testing. Details of the test performed, the genotyping results obtained and the draft report were supplied and participants were required to review the reports according to the referral information and results provided, and state whether or not each report should be authorised. If the report was deemed not fit to be authorised then reasons were required. Two reports were considered to be suitable for issuing and the majority of laboratories stated that these reports would be authorised. One report had two major errors plus two minor clerical errors and all laboratories would have not issued this report. However many comments were received regarding the format and content of the EQA reports which highlighted the changing requirements of the content of reports and the differences between laboratory reporting styles.

PM14.026

Newborn screening for cystic fibrosis (CF) using likelihood ratios derived from bloodspot Immunoreactive trypsinogen (IRT) and Pancreatitis Associated Protein (PAP)measurements

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Background
In Scotland around 350-400 DNA tests per year are required to detect around 30 cases and 35 carriers of CF. Recently, a second marker, PAP has been reported to be elevated in newborns with cystic fibrosis and a combination of IRT and PAP may provide improved specificity.
Objective
To develop an algorithm based on universal IRT measurements and subsequent PAP measurements in infants with elevated IRT levels which will maintain high detection rates but reduce the number of cases referred for DNA analysis and the incidental detection of carriers
Methods
PAP was measured prospectively by ELISA (Dynabio,France) in dried blood spots in 29 CF cases, 32 CF carriers, 314 unaffected infants with elevated IRT and 2,886 infants with IRT values <99.5th centile. IRT and PAP results were log transformed to give overlapping Gaussian distributions for the control and CF cases, from which likelihood ratios were derived for each individual IRT and PAP result.
Results
The product of the likelihood ratios can therefore be calculated thus combining the information from two markers. Setting a threshold LR of 30 as the action point for DNA testing would result in a 40% reduction in DNA testing and a 50% reduction in the number of CF carriers identified. One CF case would be missed.
Discussion
Selecting cases for DNA analysis based on subsequent measurement of PAP in infants with elevated IRT offers a cost effective method for reducing the number of DNA tests and incidental detection of carriers without the need to measure PAP in all infants.
Grant reference
Saudi Arabia government

PS14.027

Droplet digital PCR using a single primer tailed universal hybridisation probe system to confirm array CGH imbalances

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Small imbalances identified by array comparative genomic hybridisation (aCGH) require follow-up investigations to verify the finding and determine inheritance. FISH is the gold standard method used; however, this technique cannot be applied to identify small deletions and duplications. Droplet digital PCR (ddPCR) is a third generation PCR technology that allows sensitive and precise absolute quantification of copy number variations, without the need for standard curves. These qualities make this technology desirable over traditional dosage detection techniques such as quantitative PCR (qPCR).
Droplet dPCR, using Bio-Rad QX100 technology, was used to investigate the suitability of a single primer tailed universal hybridisation probe system, developed in-house, to investigate aCGH imbalances. This system can be applied to any genomic region in question, and therefore negates the need to use expensive custom Taqman probes for each region of interest, making the system versatile and affordable in a diagnostic laboratory.
All ddPCR results were consistent with the aCGH findings, and this technique was successfully applied in follow up family investigations. Furthermore, we have shown that ddPCR is sensitive to identify mosaic imbalances. To further increase affordability and to broaden the potential applications of this technique, multiplexing was also investigated, and this has been shown in principle to work using our detection method. Case studies are presented, along with cost evaluation. Future applications of ddPCR in a diagnostic laboratory are also discussed.

PM14.028

The EMQN scheme for early onset hearing loss (*DFNB1*): Facts and figures for the first six schemes.

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Early onset hearing loss is a very heterogeneous condition. However, mutations at the *DFNB1* locus can explain up to 20% of cases. The *DFNB1* locus contains two genes, *GJB2* and *GJB6*. Testing for mutations at the *DFNB1* locus has been implemented in many diagnostic laboratories because it is the first line genetic test requested by clinicians dealing with children with (prelingual) hearing loss. An external quality control scheme instigated by the European Molecular Quality Network (EMQN) started with a pilot scheme in 2008, and continued as a yearly full scheme in the following years. Here, we report on the results of the first 6 schemes.
In the annual quality control schemes, three validated DNA samples with mock clinical case descriptions were distributed to participating labs. We retrospectively analyzed the final results of each year. The number of labs participating in the last five years was on average 70. The diagnostic er-

ror rate (incorrect genotype possibly leading to a misdiagnosis) declined over the years, being the highest in 2011 (7.3%) and lowest in 2014 (1.3 %). The average genotyping score (maximum 2.00) increased from 1.76 to 1.96. However, the interpretation score varied, probably related to the perceived difficulty of the clinical questions. Overall, we have seen a general improvement in both the completeness of testing and in the reporting. This external quality control scheme is therefore a successful tool to improve the performance of participating labs and has demonstrated an improvement on reporting practice and decreasing diagnostic error rates.

PS14.029

Dissecting the diagnostic yield in clinical genomic testing

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Genomic testing has been a part of diagnosing genetic disorders since the detection of aneuploidies using cytogenetic techniques. Technological advances in high throughput, low cost DNA sequencing coupled with the availability of a high quality reference assembly have allowed us to interrogate the genome with greater precision than ever before. Together with increased understanding of the genetic underpinnings of disease, these advances mean our success rate in diagnosing genetic disease is higher than it has ever been, however the diagnostic yield of WGS/WES is only 25-50%. Improving this yield requires reexamination of the entire process, including assay development, bioinformatics analysis approaches as well as processes for robustly associating variants with disease. Assay development is critical as no single sequencing method can identify the spectrum of variant types nor can they access all regions of the genome equally. This becomes even more critical when testing for diseases that deviate from Mendelian expectations, such as cancer or diseases arising from somatic mosaicism. We are also beginning to learn that the reference assembly itself can have an impact on variant identification and interpretation. Our early, simplistic assembly models are insufficient for robust genome analysis and we must develop new models and analysis paradigms. Lastly, processes for understanding how detected variants may contribute to a particular disorder must be examined. Every person carries approximately 100 seemingly damaging variants, most of which do not contribute to rare disease. Identifying the variants contributing to specific phenotypes requires the integration of diverse biological knowledge.

PM14.030

TaqMan® Rare Mutation Assays for QuantStudio® 3D Digital PCR System

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Detection and quantification of mutant alleles in tumor tissue allow for research disease monitoring and the research of drug efficacy. Detection of emerging secondary mutations in the same tumor tissue causing resistance to potential treatment will help guide decisions on future treatment plans. A less invasive research method than using tumor tissue is testing for the presence of mutations in circulating free DNA (cfDNA).
We created a research tool for mutation detection at a sensitivity level of 1% and below. This allows researchers to find correlation between mutation types and tumor types and determination of potential secondary mutations.
The tool combines TaqMan® SNP Genotyping Assays with digital PCR. A set of assays was optimized for use in digital PCR with the QuantStudio® 3D Digital PCR System. In digital PCR, partitioning the sample into many individual reaction wells facilitates detection and quantification of rare mutant alleles. TaqMan® SNP Genotyping Assays ensure reliable discrimination of mutant and wild-type allele.
Our initial set of 38 assays covers mutations commonly found in tumor tissues: BRAF V600E, mutations in EGFR exons 19, 20 and 21, KRAS codons 12 and 13, PIK3CA exons 9 and 20, and the JAK2 V617F mutations.
All assays were wet-lab tested at a 10% mutation rate and a 1% mutation rate using mutant plasmid spiked into wild-type genomic DNA. Additionally, selected assays were tested at the 0.1% mutation rate using mutant cell lines spiked into wild-type genomic DNA.
Wet-lab results confirm that all assays showed superior performance discriminating mutant and wild-type alleles. Mutant alleles were successfully detected as low as 0.1%.

PS14.031

Determining the Limit of Detection of Rare Targets Using Digital PCR

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Detection and quantification of mutant alleles in tumor tissue allow for disease monitoring, the evaluation of drug efficacy and guide decisions on future treatment plans. Testing for the presence of mutations in circulating free DNA (cfDNA) is one of the less invasive research methods available at this time. Digital PCR presents a research tool for mutation detection in cfDNA at a sensitivity level of 1% and below.
Challenges associated with digital PCR experiments for rare allele detection include understanding the limit of detection of the assay and platform. This work compares false positive assessment strategies using the signal levels of the no-amplification cluster. Once the false call rate is established, the paper outlines a method to determine the limit of detection of the assay and platform, at a given level of confidence. The tradeoffs between sample load and sensitivity, given the number of partitions, the interrogated volume and the false call rate are also discussed.
The mathematics outlined to calculate the theoretical limit of detection is applied on a set of assays from Thermo Fisher Scientific covering the KRAS codons 12 and 13 mutations commonly found in tumor tissues. Experimental results showing a detection of at least 0.1% mutation rate are presented as examples. Test samples were created using both mutant plasmid and mutant genomic DNA mixed with wild-type genomic DNA at a predefined percentage.
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PM14.032

Dystrophinopathies: An NGS approach for the molecular analysis of DMD gene

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OBJECTIVES: DMD gene, located on Xp21, comprises 79 exons along 2400Kb, whose mutations originates Duchenne/Becker muscular dystrophies (DMD/BMD). 70% of DMD/BMD patients show deletions/duplications of one or more exons. The remaining 30% present a nonsense, missense, frameshift or splicing mutations, distributed along the entire gene. Detection of point mutations is an expensive task in terms of technical and economic issues.
The introduction of next-generation sequencing technology (NGS) in our laboratory has allowed exponentially increase sequencing throughput.
MATERIALS AND METHODS. We studied DNA samples from 60 patients with clinical suspicion of DMD/BMD who had previously ruled out the presence of an exon deletion or duplication in the DMD gene.
The generation of amplicon libraries was performed using the kit DMD MASTR of Multiplicom and they were sequenced using the MiSeq platform from Illumina. Sequencing data were analysed using Variant Studio and DNA Nexus softwares. The pathogenic changes were confirmed by Sanger sequencing and were compared with the LOVD database and Alamut and Polyphen-2 softwares.
RESULTS: We have identified 31 mutations, which have been confirmed as the cause of the pathology.
CONCLUSIONS: The analysis of point mutations in DMD gene by NGS is a breakthrough in molecular analysis time and cost, compared to Sanger technique. Thus, targeted NGS can contribute to alleviate the diagnosis delay and to overtake the genetic counseling.
In those patients in whom no mutation was identified in gDNA, the DMD/BMD clinical diagnosis should be confirmed by immunohistochemistry in muscular biopsies and afterwards the mRNA sequencing.

PS14.033

Northern Lights Assay: A versatile method for comprehensive detection of DNA damage

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Introduction: Detction of DNA damage has many applications including testing quality of DNA samples in biobanks, monitoring complex molecular procedures, genotoxicity testing, diagnosing genome instability, and in cancer theragnostics. We tested the suitability of the Northern Lights Assay (NLA) for these applications.
Materials And Methods: NLA is based on Two-Dimensional Strandness-De-

pendent Electrophoresis (2D-SDE), a technique of nucleic acid separation based on size, standness, and conformation changes induced by damage. Each specimen is analyzed in sample pairs of non-digested DNA to detect single- and double-stranded breaks and MboI-digested DNA to detect other lesions. NLA has been adapted to a microgel platform to improve sensitivity and speed of analysis. We tested NLA on various samples including DNA in solution, intermediate products of complex procedures, various cell cultures treated with genotoxic agents, and body fluid samples from patients.
Results: NLA could detect single-stranded breaks, double-stranded breaks, interstrand and intrastrand DNA crosslinks, single-stranded DNA, bulky lesions and mismatches in all types of samples. The sensitivity of the method was comparable to other methods for DNA damage detection. The main advantage of NLA compared to the comet assay was detection of both intrastrand and interstrand crosslinks and other abnormal DNA in a direct manner.
Conclusions: NLA is a versatile method for comprehensive and simultaneous analysis of various types of DNA damage both in purified DNA and in biological samples from cells and body fluids. NLA is useful in biomedical research and diagnostics including Omics technologies.
Grants: Icelandic Centre for Research. University of Iceland Research Fund, Landspitali University Hospital Research Fund and investments in Lífeind ehf.

PM14.034

Comparison of two different methods for brain DNA extraction

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Recent appreciation of the extent of mosaicism in human tissues, including the brain, implies that analysis of peripheral blood lymphocyte DNA may be insufficient. For correct assessment of CNVs, particularly mosaicism, an even genome extraction is crucial. To determine if the extraction method influences brain DNA quality and quantity, two widely used kits, DNeasy and Puregene by Qiagen, were compared.
DNA was extracted from the cerebella of three Parkinson's patients and three controls with both methods, using different starting amounts on the DNeasy for four of these. DNA was compared using Nanodrop, Qubit and electrophoresis.
DNA purity was comparable. Electrophoresis revealed a smaller main band and more smeary appearance with DNeasy. Increasing the amount of brain tissue (5, 25 and 50 mg) used with DNeasy led to a lower yield per mg brain, and a more smeary appearance. Puregene yielded up to three times more DNA per mg brain compared to the best DNeasy extractions, with yields close to the expected value based on cerebellar cell density.
Therefore DNeasy yields less DNA of worse quality per mg tissue than Puregene, and increasing starting tissue leads to lower amount and worse quality with DNeasy. Excess protein may prevent all DNA from passing through the filter, and we are concerned this might lead to different relative amounts of parts of the genome, resulting in inaccurate copy number calls. We recommend Puregene for brain DNA extractions, although we have not compared phenol chloroform, which should also be considered.
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PS14.035

Assessment of automated DNA extraction for peripheral blood, bone marrow and amniotic fluid specimens

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Introduction: Reliable of DNA extraction is a key step in molecular assay. DNA extraction using phenol-chloroform or spin-column remains the labor-intensive and time-consuming. Therefore, the automated DNA extraction is urgently needed for the routine diagnostic laboratory. The aim of this study was to evaluate the automated DNA extraction for peripheral blood, bone marrow and amniotic fluid specimens.
Materials and Methods: DNA from peripheral blood (PB), bone marrow (BM) and amniotic fluid (AF) specimens were extracted using the spin-column; QIAamp DNA blood mini kit and the automated DNA extraction; EZ1 Advanced XL. DNA concentration was quantitated by NanoDrop Spectrophotometers. For comparison of analytical performance, DNA from PB and BM were detected for mutation analysis of hematologic malignancy markers. In addition, DNA from AF was evaluated for molecular prenatal diagnosis using QF-PCR and Bacs-on-Beads technology.
Results: DNA concentration of PB and BM from QIAamp DNA blood mini and EZ1 advanced XL were 11.1 to 299.9 ng/µl and 19.1 to 108.2 ng/µl, respectively. Moreover, DNA concentration of AF from QIAamp DNA blood mini and EZ1 advanced XL were 7.5 to 19.2 ng/µl and 2.9 to 5.2 ng/µl, respectively. Analytical performance of both DNA extraction methods were concordance in all types of samples and all assay.
Conclusions: DNA concentration and quality from QIAamp DNA blood mini kit and EZ1 Advanced XL is not significantly difference. The incorporation of the automated DNA extraction into the routine DNA analysis process may reduce the need for labor-intensive, time-consuming using the spin-column DNA extractions.

PM14.036

Efficiency of Computer-Aided Facial Dysmorphology Analysis in the Medical Genetics Clinic

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Computer-aided facial dysmorphology analysis program has recently been introduced to clinicians. It has been shown (Basel et al., ASHG 2014) that the accuracy of computer-aided dysmorphology analysis is comparable to that of human experts in dysmorphology. Here, we evaluated the performance of such technology by comparing computer-aided analysis of a set of 2D facial images of patients with molecularly confirmed diagnoses.
Of a total sample of 280 frontal facial images of patients who were referred to our department processed with Face2Gene. The following criteria for selection were applied for this present study: images of patients with a molecularly confirmed diagnosis; cases with syndromes currently supported by the technology cases, with well documented dysmorphic features and proper consent.
The resulting sample for this study is a test group of 12 patients affected by 8 syndromes (Table).
A match was considered positive where the diagnosed syndrome was listed among the top ten syndromes under the "Gestalt Matches" list in Face2Gene.
In 10/12(83%) patients there was a positive match between the confirmed diagnoses and matching syndromes detected by Face2Gene. The average rank of match was 3.8 (1 being the most similar and 10 being the least).
We believe that routine use of computer-aided dysmorphology analysis in the clinic will assist to clinicians, particularly to medical geneticists, in reaching a more accurate differential diagnosis and shorten the time and the cost for achieving molecular confirmation of rare disease patients.

Common Diseases enrolled in the study.

Prader-Willi Syndrome; PWS
Wolf-Hirschhorn Syndrome; WHS
Rubinstein-Taybi Syndrome
Chromosome 22q11.2 Deletion Syndrome
Down Syndrome
Achondroplasia; ACH
Williams-Beuren Syndrome; WBS
Trichorhinophalangeal Syndrome

PS14.037

Use of an augmented exome for disorders with high genetic heterogeneity

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As exome sequencing enters clinical care, a dilemma in testing for disorders with high genetic heterogeneity arises: to order a gene panel test, or exome sequencing? Core to this dilemma is that the sensitivity of each approach is compromised, albeit differently. While the analytical sensitivity for genes on a panel tends to be high, the *diagnostic* sensitivity of such tests can be compromised through failure to include some genes associated with the condition. In contrast, while conventional exome sequencing assays more genes, it suffers from issues of analytical sensitivity, e.g. poor coverage of known disease genes, which in turn compromise diagnostic sensitivity.
To address this dilemma, an augmented exome sequencing assay, the ACE Clinical Exome Test, was developed. Coverage of >8000 biomedically relevant genes is enhanced, with >6000 considered "finished" (>99% of bases covered at 20x). To further improve sensitivity, the test includes coverage of interpretable non-exonic regions and genome-wide detection of structural variants.
In cases involving disorders with high genetic heterogeneity, current gene

panel tests were examined to determine if they included the disease-associated genes identified through augmented exome sequencing. This revealed that the genetic diagnoses of several individuals would have been missed through gene-panel testing. Examples including cases of Charcot-Marie-Tooth disease and Leber congenital amaurosis are presented.
For disorders with high genetic heterogeneity, an augmented exome test that addresses the issues of analytical sensitivity associated with conventional exome sequencing, can provide diagnoses that may be missed through gene panel testing, and simplify the panel vs. exome test-selection dilemma.

PM14.038

Coverage Analysis of Lists of Genes involved in Heterogeneous Genetic Diseases following Benchtop Exome Sequencing using the Ion Proton

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Exome sequencing has been proven efficient for clinical applications in heterogeneous genetic diseases, including exome sequencing associated with data filtering for selected genes. However, previous reports have been based mainly on the use of large-scale Next-Generation-Sequencing platforms, difficult to implement in medium-scale genetic diagnosis laboratories. In this context, the Ion Proton™ (Life Technologies, CA, USA) allows for facilitated implementation of exome sequencing in medium-scale laboratories, through an easy set-up in 6 hour runs on a benchtop apparatus producing high quality exome data.
As a requirement for diagnostic mutation screening using Ion Proton™ exome sequencing, we precisely evaluated sequence coverage data for six groups of genetically heterogeneous diseases, of interest for genetic diagnostic laboratories: myopathies (82 genes), hereditary motor and sensory neuropathies (55 genes), early onset epileptic encephalopathies (30 genes), isolated and combined dystonia (12 genes), non syndromic deafness and hereditary hearing loss (60 genes), and intellectual disability (107 genes for X-linked transmission; 39 genes for autosomal recessive transmission, 37 genes for autosomal dominant transmission). In addition, we evaluated sequence coverage for a list of 57 genes for which the ACMG recommends to report incidental findings.
The simple workflow of Ion AmpliSeq™ exome enrichment combined with Ion Proton™ simplex-sample sequencing allows for >90% mean sequence coverage at ≥20X for approximately two-third of the genes of interest analyzed for the disease-groups myopathies, hereditary motor and sensory neuropathies, early onset epileptic encephalopathies, non syndromic deafness and hereditary hearing loss, and intellectual disability (X-linked, autosomal recessive, and autosomal dominant forms), and the list of genes for which the ACMG recommends to report incidental findings.

PS14.039

The translation of exome sequencing into diagnostic genomics

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Introduction: Until recently, the sequencing of whole exomes for the diagnosis of genetic disease remained largely in the realm of the research community. We investigated the efficacy and application of such techniques in clinical practice.
Materials and Methods: Whole or focussed exome sequencing was performed on a cohort of 93 mixed referrals using the Sureselect (Agilent) capture reagent and Illumina HiSeq sequencing platform. Two different in-house analysis pipelines were applied (dependent upon genetic heterogeneity of diagnosis), designed to maximise sensitivity and diagnostic efficiency, respectively. Filtering of variants was performed based on phenotypic gene lists, coding sequence location and predicted effect, and minor allele frequency.
Results: An overall diagnostic yield (definitely- or likely-pathogenic variants) of 42% was achieved across a range of referrals, including large sub-groups of ciliopathy and aortopathy patients, and a further 12% of cases had potentially-pathogenic variants warranting further follow-up. Horizontal and vertical coverage were superior on focussed versus full exomes, however, full exome analysis was used to identify a presumptive new gene (for primary ciliary dyskinesia), and facilitated re-analysis of data in heterogeneous conditions with rapidly-expanding lists of genetic causes. Exonic copy-number analysis using NGS data was validated, and two causative large deletions were detected. No significant incidental findings were identified.
Conclusions: Exome sequencing is a flexible and highly efficient method for mutation detection in a range of diagnostic scenarios, and promises to revolutionise laboratory diagnosis in the genomic era.

PM14.040

The clinical utility of exome analysis in the routine clinic.

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Introduction: The Oxford Medical Genetics Laboratory and the Oxford Biomedical Research Centre are working in collaboration to translate whole exome sequencing (WES) into routine practice. This project has three main objectives: first, to reaffirm the clinical utility of exome sequencing and develop pathways for data analysis, and clinical reporting; Second, to establish an ethically approved framework for patient referral, analysis and results reporting; and third, to transition WES into a diagnostic service.
Materials and Methods: To date >190 exomes (28 Trios, 14 affected sibs, and 78 probands) have been reviewed and approved for WES by a Genomic Medicine MDT. The initial data analysis focused on known causative genes, followed by whole exome analysis with appropriate consent.
Results: Molecular confirmation of a highly likely or likely pathogenic variant was reported in ~29% of cases. ~4% of cases had a variant of unknown significance reported in a clinically relevant gene. A number of these cases highlight a short fall in previous analysis methods. We present four cases illustrating the clinical efficacy of WES across four different disorders: primary immune deficiency, neonatal cardiomyopathy, ectodermal dysplasia, and a neonatal dysmorphic disorder. These cases demonstrate how virtual gene panels allow the rapid integration of recently discovered disease-causing genes, which can have a profound impact of patient management. In addition, three of these cases demonstrate how WES influenced or outperformed established diagnostic services.
Conclusion: Our data support a focused exome in the first instance; however, WES can provide greater clinical sensitivity and influence existing clinical practice.

PS14.041

Exome Triage: Strategies for computational rescue when analysis in the clinical search space fails

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In 2008, the National Institutes of Health (NIH) began the NIH Undiagnosed Diseases Program (UDP), whose purpose is to find diagnoses and conduct research for study participants who remain undiagnosed despite an extensive medical workup. Approximately 150 patients are admitted to the UDP per year, at which point they undergo a robust clinical evaluation and, when warranted, an in-depth genomic analysis. Since clinical exome sequencing has become incorporated into the standard armamentarium for evaluating complex patients, the UDP has increasingly needed to focus on detecting disease-causing DNA variations in regions that are missed using standard exome techniques. To this end we have created an analytic pipeline that combines standard methodology with enhancements designed to detect and/or recover variants in where standard pipelines may generate false negative results. The enhancements include such strategies as parent-aware diploid alignment, aggressive use of high-density SNP array data, and standardized-phenotype-based variant prioritization. Over time, our aim is to refine our protocols for automated and manual analysis to create a suite of "second pass" strategies for cases where standard clinical exome and genome studies have not yielded actionable results.
This research is supported by the Intramural Research Program of NHGRI and the Common Fund of the National Institutes of Health.

PM14.042

Is exome sequencing of single patients with intellectual disability an effective diagnostic strategy?

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Trio-sequencing can be used in all disorders, and has proven its value in

finding causes of intellectual disability (ID). We investigated whether sequencing only the affected patient without parents is sufficient to find the causative mutation, leading to a considerable reduce in costs. In this study, we enrolled 36 patients with unexplained ID, and sequenced the exome. The exome sequences were analysed with a stringent post-sequencing annotation pipeline including an ID gene panel of ~500 genes for filtering of the data. All remaining variants with a potential clinical consequence were validated by Sanger sequencing and tested in the parents for inheritance.
After variant filtering we noticed an average of 13 variants per patient (range 2 to 27) requiring further clinical interpretation. The majority of these variants were inherited from one of the parents. Hitherto, we identified 5 de novo mutations and 1 homozygous mutation in 33 patients (18%). For the remaining 27 patients both parents have been sequenced and further analysis is being performed.
Without exome sequencing the parents, a relatively high amount of potentially pathogenic variants remain. All these variants require clinical interpretation which is very time-consuming, while most of these variants were likely benign because they are inherited from one of the parents. With trio-analysis inherited variants can be filtered out suggesting that this strategy, at this moment, is more efficient in identifying the causative variant. In the future when databases are filled with more and more exome data and consequently with more rare benign variants, exome sequencing single patients will become a more realistic diagnostic approach.

PS14.043

Reshaping genetic diagnostics of rare diseases with transition to genome-wide sequencing approaches - experience of Slovenian centre for Mendelian genomics

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Establishment of diagnosis in patients with suspected genetic disorder presents a challenging task, mainly due to extensive diversity and rarity of genetic conditions. Aiming to improve the accessibility to genetic testing, enhance the diagnostic yield and rationalize genetic testing in Slovenia, we implemented clinical exome sequencing as routine mode of genetic testing for a wide variety of genetic disorders. Our experience offers unique insight into clinical effectiveness of such strategy in daily clinical practice.
We performed a comprehensive analysis of patients referred since 2013, including data on referral diagnosis, disease classification, ontological phenotype characterization and other clinical parameters. For each patient, we evaluated the outcome in light of initial diagnosis, distinguishing cases where the result either recapitulated initial diagnosis, established diagnosis in undiagnosed cases, or resulted in diagnosis reclassification. We additionally monitored added value of extended exome analyses, including detection of CNVs, mitochondrial variants and exome-based linkage analysis.
Overall, sequencing in 347 diverse patients revealed convincingly causative genetic variants in 139 (40.1%), with substantial variability across different disease categories. Causative variants were found in referred gene panels in 30.1%, while establishment of new diagnosis was reached in 6.9% and reclassification in 1.7% of cases. In 9 cases, causative variant was identified utilizing extended methods of exome data analysis.
In conclusion, we demonstrate the multifaceted efficacy of clinical exome sequencing in diagnostics of genetically and phenotypically diverse diseases. Furthermore, we show its implications in widening national accessibility of genetic testing to thousands diseases, consequentially reducing the need for cross-border genetic testing.

PM14.044

Reducing diagnostic turnaround times of exome sequencing for families requiring time effective diagnostics

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Whole exome sequencing (WES) has now entered the medical practice with powerful application in the diagnosis of rare mendelian disorders. Although the usefulness and cost-effectiveness of this diagnostic test is widely demonstrated, there is a critical need for reducing the results turnaround time to make WES a clinical reality. Since 2011, automation of laboratory procedures and advances in sequencing chemistry made possible a ~50-hours diagnostic whole genome sequencing from the blood sample to molecular diagnosis of suspected genetic disorders. Taking advantages of these advances, the main objective of the study was to improve the sequencing results turnaround times. WES was proposed to 18 patients with severe undiagnosed disorders with developmental anomalies, facing a medical situation requiring urgent diagnosis. Each family gave consent and new specimens were gathered. The extracted DNA was sequenced on NextSeq500 (Illumina) instrument. Data analysis was performed following standard procedures. Variants interpretation was performed using in-house software. Each rare variant affecting protein sequence with clinical relevance was tested for familial segregation. Overall, 4.4 gigabase of sequence were produced per individual, resulting in a mean coverage of 93 folds, with 93% of the RefSeq exons covered by at least 10 reads. Diagnostic rate was of 39% (7/18), with a maximum turnaround time of 8 weeks from specimen arrival to results. Direct impact of a rapid diagnostic for positive families included two prenatal testing proposals, two clinical trials inclusions. Finally, this pilot study demonstrates the feasibility of reducing the turnaround times for diagnostic WES in our primary genetic center.

PS14.045

What does exome sequencing miss? An exploration of the false-negative space in current exome sequencing

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Exome sequencing has advanced to clinical practice and has proven to be useful in the molecular diagnosis of rare genetic diseases. At the NIH Undiagnosed Diseases Program we routinely apply exome sequencing in the pursuit of disease causing variants in patients that have not been able to receive a diagnosis despite extensive medical workup. An increasing number of our new patients present with prior, negative clinical exome testing. In these cases, given the hypothesis of a genetic condition, the disease-causing variant may be hidden beyond the search space defined by current exome sequencing practice. Such false negatives may be caused by a number of factors including specific decisions made during data analysis and limitations in the basic technology. We present data from a literature review, plus examples from our own datasets where possible, to describe and quantitate the false-negative space associated with current practice. Examples include allele skewing, false *de novo* calls caused by incorrectly called parental genotypes, mis-alignment, mapable but low coverage regions, non-mapable targeted regions, misalignment and indel errors. Understanding the false-negative space associated with clinical exome sequencing provides a rational basis for future work on methods for improving detection in systematically under-assayed genomic regions.
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PM14.046

Genetic diagnosis of familial hypercholesterolaemia in the UK and Ireland using the Randox FH Biochip array

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Introduction: Familial Hypercholesterolemia (FH) is a genetic disorder characterized by high levels of low density lipoprotein in the cardiovascular system and early onset of cardiovascular disease. FH is most commonly caused by mutations ApoB, LDLR, and PCSK9 genes. FH can be treated quite effectively with lipid-lowering drugs and lifestyle changes. Genetic testing and cascade screening have been recommended by the National Institute for Health and Clinical Excellence (2008). We have developed an assay, based on a combination of multiplex PCR and biochip array hybridization, which enables the rapid simultaneous detection of 40 mutational targets within ApoB, LDLR and PCSK9. The assay detects 71% of all point mutations in FH patients within the United Kingdom (based on 465 families from a variety of ethnic backgrounds with identified FH mutations).
Materials and Methods: A total of 500 patients which met the Simon Broome criteria for possible FH were referred from across the UK for genetic scree-

ning. Testing was carried out using the FH Array biochips and the Randox Evidence Investigator analyzer. Results were processed automatically, with analysis completed within 3 hours from template DNA.
Results: Mutations were detected in 6.4% (32/500) patients tested. These consisted of 29 heterozygous LDLR mutations, 2 familial defective ApoB mutations and a patient who was compound heterozygous for ApoB R3527Q and LDLR R350X.
Conclusion: The FH array successfully identifies the most prevalent mutations in the UK and Ireland. It will aid confirmation of suspected FH cases and in cascade screening, hence reducing FH associated morbidity and mortality.

PS14.047

Development of a novel targeted panel sequencing assay to identify patients with Familial Hypercholesterolemia

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Introduction: Familial hypercholesterolemia (FH) is a common autosomal dominant disorder of lipid metabolism. Genetic diagnosis of FH currently includes the study of LDLR, APOB (2 fragments of exons 26 and 29) and PCSK9, by PCR plus Sanger sequencing. We present the development of a novel targeted panel sequencing (TPS) approach for the genetic diagnosis of FH.
Material & Methods: A TPS panel (LDLR, APOB, PCSK9, LDLRAP1, APOE) was developed using an Ilumina MiSeq platform. Design experiment uses Long Range PCR to amplify 14 different fragments: LDLR (45.66kb), APOB (43.85kb), PCSK9 (26.51kb), LDLRAP1 (26.51kb), APOE (4.84kb). Library preparation was conducted using Nextera®XT DNA Sample Preparation Kit (Illumina). Libraries were pooled and sequenced using paired-end 2x250bp reads in a single sequencing run.
Results: To test this novel approach 16 samples have been sequenced. A total of ~1.28M reads were obtained with 40.000-155.000 reads/sample. The total percentage of bases with >=Q30 was 85.5%. Variants identified by TPS will be presented and compared with those obtained by Sanger sequencing.
Discussion: Due to the recently reports of novel APOB functional mutations outside the currently studied regions and APOE deletions causing FH, it is imperative to change the FH genetic diagnosis. This TPS assay includes flanking intronic regions, may be easily expanded to include additional genes and offers a faster delivery of results compared to the traditional sequencing approach. The 424 FH mutation negative (by current genetic diagnosis) index patients, in our FH cohort, will be analysed using this novel TPS approach for patient identification improvement.
National Institute of Health Doutor Ricardo Jorge research grant [BRJ-DPS/2012]

PM14.048

Accurate quantification and qualification of FFPE samples increases success rate of NGS library prep and sequencing

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FFPE tissue archiving is the most widely used method for clinical sample preservation, and provides a valuable source of diverse genetic information for cancer biomarker discovery. DNA recovered from FFPE tissues exhibits varying degrees of fragmentation, cross-linking, deamination, depurination and other lesions due to formalin fixation, paraffinization, and storage conditions; and as a result, NGS library preparation is often challenging. To increase success with FFPE-derived DNA, we developed a qPCR-based method to determine quantity of amplifiable DNA and extent of degradation. SureSelect XT libraries were prepared from multiple FFPE samples of varying integrity, and enriched using the ClearSeq Comprehensive Cancer Panel which targets 151 genes frequently mutated in solid and hematological cancers. As we will show, qPCR integrity scores are highly correlated with pre-capture PCR yield and sequencing metrics (library complexity and coverage), and can be used as a guide to determine appropriate sequencing depth for optimal coverage.

PS14.049

SuperScript® IV Reverse Transcriptase: A New Reverse Transcriptase for RNA Analysis

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Survey and interview studies conducted over a three year period revealed that researchers are not satisfied with their current reverse transcriptase and are performing reactions with increasingly difficult samples, such as poorly purified RNA that contains inhibitors and unpurified RNA (direct RT). To meet this performance gap, the Thermo Fisher Life Sciences Solution group combined technologies from the former Thermo Fisher Scientific and Life Technologies to produce a new reverse transcriptase. SuperScript® IV is the first reverse transcriptase enzyme launched from joint efforts and experimental evidence shows that it is the most robust reverse transcriptase compared to other enzymes. SuperScript® IV characterization was performed in the context of "real world" situations where users do not have perfect RNA samples. Using a variety of stringent assays, we demonstrate that SuperScript® IV possesses superior performance in a variety of inhibitors, such as alcohols, salts, detergents, phenol, heparin, hematin, bile salts, and formalin typically found in sample preparation reagents, cell lines, blood, feces, and FFPE samples. This enzyme can even detect RNA targets in unpurified RNA samples (directly lysed cells) and whole blood without sacrificing sensitivity and yield. The introduction of SuperScript® IV enables researchers to obtain more consistent results independent of sample quality and simplify and speed up workflows by eliminating RNA purification.

PM14.050

Data sharing and interpretation amongst Dutch genome diagnostic laboratories

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The interpretation of the vast amount of DNA variants NGS produces is a huge challenge. Sharing of detected variants between laboratories would be of great assistance to differentiate pathogenic and benign DNA variants. Moreover, dozens of annotation tools and databases, including tissue/cell/allele specific gene expression, QTL and GWAS associations, drug targets, pathogenicity prediction toolss, model organism studies, metabolic/signalling pathways, clinical actionability, are emerging to aid variant classificatio,. What are the best practice protocols and tools?
To address these challenges, the Dutch Society for Clinical Genetic Laboratory Diagnostics (VKGL) has started the NGS data sharing working group. Clinical diagnostics labs can easily upload individual VCF files to the national data sharing server that are then aggregated for anonymous public queries. In addition, we have built the MOLGENIS.org system so that bioinformaticians can now plug-in new annotation scripts, optimal data storage solutions (e.g. VCF, local databases, search indexes) and visualizations for rapid community evaluation.
Clinical labs can now easily interrogate patient mutations observed in other labs. We expect this effort to develop into a valuable NGS data exploration app as well as a sharing platform for best practice data and pipelines, integration with international sharing platforms such as GA4GH and Cafe Variome (for which pilots are underway), well-curated reference knowledgebases, and optimal user interfaces, results of which can disseminate into research institutes, clinical software companies and individual labs.

PS14.051

Streamlining NGS workflows by the application of the DNA Integrity Number (DIN) from the Genomic DNA ScreenTape Assay

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The results of numerous molecular screening and assay methods often rely on the overall quality of the genomic DNA (gDNA) input material. However extraction of genetic material can be challenging and often results in low amounts or variable quality of gDNA samples, which are further subjected to time and cost intensive downstream applications. For example, array comparative genome hybridization (aCGH) and Next Generation Sequencing (NGS) can require intact, high quality gDNA to ensure high quality, unambiguous results. It is therefore widely recommended to perform an initial quality control (QC) of the input material. Especially as only the final step of these workflows reveals if meaningful results have been achieved. In order to provide an automated measure for gDNA integrity assessment, a software algorithm has been developed. This functionality of the 2200 TapeStation system provides a numerical determination of gDNA integrity and is referred to as the DNA Integrity Number (DIN). The data presented here demonstra-

tes how the standardization of sample integrity assessment using the DIN allows the establishment of workflow specific thresholds for the upfront QC of gDNA on the Agilent Genomic DNA ScreenTape assay. In this study the objective determination of the sample integrity resulted in significant saving of sequencing and sample preparation overhead in NGS workflows.

PM14.052

Melbourne Genomics Health Alliance: evaluating a whole of system approach to implementation of genomics into clinical practice

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To optimise the use of genomics as part of routine practice, whole of system change is needed. Melbourne Genomics Health Alliance is clinically driven, addressing change in health, diagnostic, workforce and information management across seven healthcare, research and academic organisations. In the demonstration phase, barriers are identified and resolved. Adults and children (n = 250) with one of five germline or somatic conditions are being offered whole exome sequencing with targeted analysis. Importantly, this is in parallel with standard investigations. Evaluation is being performed on the process and outcomes of counselling, diagnostic testing, multidisciplinary review, reporting, data linkage, patient entry of lifestyle data and ease of access to (raw) data by researchers. Data collected includes feedback from clinicians and participants. Initial results (n=144) demonstrate a 30% diagnosis rate overall; performance relative to standard care is variable across conditions. A key feature of the project has been demonstrating that common standards for exome sequencing, curation and interpretation can be agreed and applied across different diagnostic laboratories. The common, highly-automated bioinformatics pipeline and linked database for curation of filtered variants have been well received by those performing curation. Multidisciplinary meetings to interpret results have proven a highly effective means for engagement and education. Participant expectations differed between adult participants and parents of a child participant. Data linkage has expanded the information available for research and clinical care and laid the foundation for data sharing to organisations outside the Alliance. Detailed results of the outcome and process evaluation will be presented.

PS14.053

Clinical genome analysis: delivering the right diagnosis

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Whole genome sequencing and analysis (WGA) presents an unprecedented opportunity to transform clinical genetic services for patients with rare diseases. Yet converting whole genome data into clinically useful results is not an uncomplicated procedure. This unique project set out to influence policy, by informing policy-makers of the challenges of embedding genome sequencing in health services for patient benefit. Through consultation with a group of experts including clinical geneticists, clinical scientists and molecular scientists, we examined different aspects of the genome analysis process in the context of rare disease diagnosis to (i) explain the barriers that need addressing in order to use genome sequence data for clinical purposes, and (ii) present the changes in policy required to enable the safe and effective application of genome sequencing in healthcare. Our examination has highlighted the following challenges when applying genome analysis within a clinical diagnostic pathway: the integration of bioinformaticians within the healthcare workforce; the development and application of standards and best practice guidelines to WGA; improving the evidence base on which data analysis and interpretation depends; and optimising the mechanisms for phenotyping patients and capturing this information to support efficient WGA. These challenges to achieving accurate and effective WGA are not insurmountable, but overcoming them will take resources, commitment from stakeholders, and a methodical yet proportionate approach. Realising the benefits of WGA will require: establishing best practices in bioinformatics; determining analytic standards; improving data and knowledge exchange; establishing adequate computational infrastructure to perform genome analysis and interpretation and transfer of data.

PM14.054

Sporadic hereditary motor and sensory neuropathies: advances in the diagnosis using Next Generation Sequencing technology

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Hereditary motor and sensory neuropathies (HMSN) are genetically heterogeneous disorders affecting peripheral motor and sensory functions. Many different mutations in several HMSN causing genes involved in the demyelinating, the axonal and the intermediate form have been identified, for which all inheritance patterns have been described. The mutation screening currently available is based on Sanger sequencing and is time-consuming and relatively expensive due to the high number of genes involved and to the absence of mutational hot spots. To overcome these limitations, we have designed a custom AmpliseqTM panel for simultaneous sequencing of the coding DNA sequence (CDS) of 28 HMSN-related genes. We have applied this panel to three representative patients with variable HMSN phenotype and uncertain diagnostic classification because of an uninformative family history and electrophysiological findings suggestive but not fully conclusive for axonal, demyelinating or intermediate form. Using our NGS platform we rapidly identified three already described pathogenic heterozygous mutations in MNF2, MPZ and DMN2 as disease-causative mutations in the three cases, respectively. Here we show that, while utilization of traditional molecular techniques would be time-consuming and expensive, our pre-custom platform allows a fast, specific and low-cost diagnosis in sporadic HMSN cases. This prompt diagnosis is extremely useful for providing a well-timed treatment, establishing a recurrence risk and preventing further investigations poorly tolerated by patients and expensive for the health system. Importantly, our study illustrates the utility and successful application of NGS to mutation screening of a Mendelian disorder with extreme locus heterogeneity.

PS14.055

Towards a uniform nomenclature to improve the patient reports and databases: results from two pilot EGFR External Quality Assessment schemes

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Introduction. Uniform reporting of mutations in an electronic database is essential. Laboratory databases as well as electronic medical records will be contacted in the future, to create large reference databases for health benefit research. The Human Genome Variation Society (HGVS) nomenclature guidelines are the golden standard for an unambiguous and uniform reporting system for mutations.
Materials and Methods. Diagnostic reports of two ESP external quality assessment schemes for *EGFR* mutation analysis were analyzed in detail regarding the nomenclature.
Results. The *EGFR* mutation was correctly identified and reported in 81 reports in 2012 and 137 reports in 2014. The number of reports with nomenclature according to the most recent guidelines of HGVS were, respectively for 2012 and 2014, 32.1% and 28.5%. The most used error, was the use of traditional nomenclature (e.g. G719S), respectively 38.3% in 2012 and 54.7% in 2014. Reporting on both DNA and protein level was done by 67.9% in 2012 and only 59.1% in 2014. The number of labs reporting a three-letter code for each amino acid has decreased from 39.5% in 2012 to 31.4% in 2014, of which respectively 8.6% and 13.9% also report the one-letter code in parallel.
Conclusion. The results show the need for improvement of mutation reporting according to HGVS guidelines. From 2014, points are deducted for non- or incorrect HGVS nomenclature in order to increase the attention of laboratories for a correct and unambiguous reporting of mutations. Further research will be performed with nomenclature results from different EQA providers.

PM14.056

Full length HLA genotyping using Pacific Biosciences SMRT sequencing

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HLA genotyping through sequence-based typing (SBT) is subjected to phase ambiguity and genotyping uncertainties, leading to delays in patient/donor typing. Herein, we summarise our experiences in setting up a high-throughput genotyping approach using Pacific Biosciences (PacBio) SMRT-sequencing as a means to provide full length HLA sequences for adequate phasing and identification of novel alleles within a clinical setting.

Locus-specific amplification of HLA Class I and II loci produced full-length amplicons encompassing the enhancer/promoter region to the 3'UTR. Following PacBio SMRT-sequencing, single-molecule sequences were analysed via the long amplicon analysis pipeline and genotyped using third-party genotyping software (n=12). Initial results revealed full concordance with the anticipated SBT results. Both alleles from both heterozygous and homozygous individuals were completely resolved and formerly ambiguous alleles were readily determined. A number of novel intronic sequence variants were also discovered.
Given the quality of the data generated, it was clear the sequencing of full-length HLA genes using PacBio approach could accommodate a high degree of multiplexing. Consequently, we are conducting a pilot study (n=50) in association with the Fred Hutchinson Centre to develop a streamlined pipeline from sample collection to genotype acquisition. Within this pilot we are evaluating optimal primer sets, level of multiplexing, automation of laboratory and data analyses steps and the use of third-party genotyping software. Preliminary results reveal that PacBio sequencing will allow for high-resolution and high-throughput sequencing of full-length HLA loci with a fast turn-around time. However, further optimisation of the pipeline is needed.

PS14.057
Genotyping of alpha thalassaemia mutations by high-resolution DNA melting analysis

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Alpha-thalassaemia is an inherited globin gene disorder commonly found among the Greek population, composed of both non-deletional and deletional alpha-globin gene mutations. In Greece, 7% of the general population is carrier of α-thalassaemia (deletion 3.7 kb-α3.7) while 1% carries α^0 deletions or rare point mutations an (non-deletional, α^T).
The high prevalence (8%) and heterogeneity in molecular level, makes necessary the development of a reliable, cost effective and rapid scanning method for alpha globin gene analysis, easily adapted to a routine laboratory.
Here we describe the development of an alpha thalassaemia specific High Resolution Melting Analysis (HRMA) approach. Specific sets of primers were designed to cover eight regions of the HBA genes containing the most frequent hitherto reported mutations of the Greek population [$\alpha2^{Agrinio}$, $\alpha2^{Hph}$, $\alpha2^{Icaria}$, $\alpha2^{CS}$, $\alpha2^{PA1}$, $\alpha2^{PA2}$, α2 cd115(+CC)]. PCR conditions were designed identical for all amplicons, permitting thus multiplexing. Furthermore, Real-Time GAP-PCR was performed in order to identify deletions (-α3.7, --med, -20.5, -α4.2).
Initially, 75 previously genotyped samples (heterozygotes or homozygotes) were analysed. Different mutations produced distinct derivative plots when subtracted from the reference curve of a wild type control, resulting in 100% accurate mutation identification. In addition, HRMA analysis of 125 undefined samples totally matched subsequent diagnosis by either Sanger sequencing or ARMS analysis.
HRM curve analysis is rapidly becoming the prominent mutation scanning methodology representing a rapid, simple, cost-effective and highly feasible strategy for identifying effectively underlying mutations. We successfully performed HRMA for alpha-thalassaemia in order to identify frequent α-thalassaemia mutations in terms of carrier screening and prenatal diagnosis.

PM14.058
Correct result, but was the correct sample tested? Introducing the UK NEQAS for Molecular Genetics Tissue Identification EQA Scheme.

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Objectives
To provide an EQA for the molecular testing of FFPE specimens to determine identity.
Incidents occur in laboratories which result in doubt over the origin of tissue samples. Therefore it is necessary for laboratories to test samples for confirmation of the origin of the tissue, detect sample switches or identify sample mislabelling. This often aids in the delivery of a patient result and minimises the need for re-sampling.
Laboratories test formalin fixed paraffin embedded (FFPE) tissue samples using molecular fingerprinting assays. To provide laboratories with assurance of the quality of this testing and accuracy of data analysis, a pilot external quality assessment (EQA) was offered to laboratories during 2015.
Laboratories were supplied with three EQA scenarios with accompanying rolled sections of FFPE tissue (1 x 10μM curled section per sample) and were required to extract DNA and test according to their usual procedures. The results were to be interpreted with respect to the scenario provided and to determine the likelihood that the multiple tissue samples supplied were from the same patient. To ensure the tissue samples were appropriate for testing by molecular fingerprinting assays all EQA samples were validated by two independent laboratories using different assays. The results were as expected and gave high quality reportable molecular test results. Fourteen laboratories participated in the pilot EQA and the reported genotyping results, interpretation of the data and the methodologies used in the EQA run will be presented.

PS14.059
The clinical utility of QF-PCR microsatellite analysis in sample identification and patient management

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An in-house QF-PCR microsatellite assay, used routinely for the prenatal diagnosis of chromosomal aneuploidy, has been re-optimised and applied in the identification and quantification of contributing genotypes within FFPE tissues. Microsatellite analysis has been used successfully to answer clinical questions arising from suspected clinical incidents; preventing patient misdiagnoses and mismanagement.
Here we present 2 cases where the results of microsatellite analysis played a crucial role in patient management. Case ONE details how microsatellite analysis of FFPE tissue and reference peripheral blood samples was not only used to prove that a patient panel of prostate core biopsies, as suspected, had been wrongly labelled, but also that of the batch of 4 patients' samples processed along-side the mislabelled sample, 2 further samples had been mislabelled. Comparison of patient prostate and reference genotypes facilitated the correct identification and assignment of all patients' prostate core biopsy samples. Case TWO details how microsatellite analysis of FFPE material was used to confirm the clinical suspicion that histologically malignant and normal tissues samples from 2 individuals had become embedded in a single resection block. Microsatellite analysis of macro-dissected FFPE material from different areas of a resection slide showing a 'mix' of tissues and comparison to a reference patient sample genotype confirmed the presence of 2 genotypes, consistent with sample mixing, preventing inappropriate clinical management.
Tumour identification services are now being offered by the Laboratory Genetics Service for Wales, Cardiff.

PM14.060
Targeted next generation sequencing a powerful tool to improve the care of blind infants.

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Introduction: Leber congenital amaurosis is the earliest and most severe retinal dystrophy, and a leading cause of blindness in children. The visual outcome of affected infants is variable, ranging from light perception, to low but measurable visual acuity in the first two decades of life. Blind infants with LCA are at risk of developing skeletal, neurologic and renal dysfunctions. Both the visual and extraocular outcomes strongly correlate with the disease-gene. The study presented here aimed at assessing targeted NGS (T-NGS) as a tool to improve patient care by allowing efficient and early molecular diagnosis in infants with severe visual deficiency.
Methods: 260 index LCA cases were sequenced using a T-NGS array comprising 45 genes causing isolated or syndromic LCA and 10 genes of differential diagnoses. Mutations were confirmed by Sanger sequencing and familial segregation analysis.
Results: We identified causative mutations, including 6 copy number variations, in 158/260 index cases, 130/158 of whom had mutations in genes for isolated LCA/EOSRD and 15/158 in genes for syndromic forms. In addition, we identified convincing mutations in genes for differential diagnoses in 13/260 individuals.
Conclusions: T-NGS molecular diagnosis proved powerful to improve the care of infant with severe visual dysfunction by allowing i) early identification of children with differential diagnosis which outcome is highly favorable compared to LCA (13/260; 5%) and, ii) early discrimination of children at no risk of being affected with syndromic LCA (130/158; 82%) from those,

far fewer, at risk of developing extraocular symptoms (15/158; 9%) who require extraocular explorations.
Grants : Retina France, INSERM

PS14.061
Identification of rarehemoglobinopathy by matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF/TOF)
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Thalassemia and hemoglobinopathy is the public health problem in Southeast Asian population. Rare hemoglobinopathy is occasionally found from routine screening using High-Liquid Performance Chromatography (HPLC) or suspected in people with low mean corpuscular volume (MCV) and low mean corpuscular hemoglobin (MCH) of red blood cells with no obvious identified peak from HPLC. Here we develop MALDI-TOF/TOF method to detect silent hemoglobinopathy, which cannot be detected by HPLC. In addition, we also use this technology to detect the mass of rare unidentified hemoglobin (Hb). DNA sequencing of alpha and beta globin chains was done in parallel to establish molecular diagnosis.In 2013-2014, our Genetics Service received several referrals for families carrying unidentified Hb. We can identify Hb Cheverly[beta 45(CD4) Phe>Ser] and Hb La Desirade[beta 129(H7) Ala>Val] in families with unknown causes of low peripheral oxygen saturation and normal HPLC. HbDhonburi[beta 126(H4) Val>Gly]was discovered in a family with slight increased Hb A_2 suggestive of beta thalassemia carrier. Hb New York [beta 113(G15) Val>Glu], Hb Louisville [beta 42(CD1) Phe>Leu]and Hb J-Buda [alpha1 61(E10) Lys>Asn]were identified in families with unknown peak of hemoglobin performed by HPLC. However, MALDI-TOF/TOF failed to identify the mass of some Hb such as Hb E[beta 26(B8) Glu>Lys]and Hb Zurich-Langstrasse[beta 50(D1) Thr>Ser]. In conclusion, MALDI-TOF/TOF is the potential high-throughput analytic method to identify abnormal globin chain with mass difference approximately 20 Da mass.

PM14.062
Method for fast screening of Y chromosome microdeletions by multiplex real-time touchdown PCR coupled with melting curve analysis
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Male infertility accounts for 40-50% of couple infertility and represents a complex reproductive health problem with a substantial genetic basis and various environmental risk factors. Microdeletions in the AZF regions of the Y chromosome represent the most frequent molecular genetic cause of severe infertility accounting for 15% of cases of non-obstructive azoospermia or severe hypospermatogenesis.
The aim of this work was to further develop a fast and accurate screening method based on multiplex real-time touchdown PCR followed by melting curve analysis to scan AZF loci looking for microdeletions.
The European 2014 best practice guideline for molecular diagnosis of Y-chromosomal microdeletions were used as a basis for designing five real-time multiplex PCR systems, in which the selected combinations of 24 STS amplicons can strikingly be identified by their melting points with EvaGreen™ as the high-performing DNA intercalating dye.
In order to validate our testing method we analysed 120 azoospermic and oligozoospermic males previously tested by independent singleplex PCRs. A group of 130 healthy men as positive controls and 10 women as negative controls were also included in this study. We precisely detected the absence/presence of STS in our patient group of infertile men based on our method. No deletions were detected in the group of healthy men and no amplification for any of the STS tested were observed in the negative control group.
Our method allows a fast, simple and low-cost screening for Y chromosome microdeletions in patients with genetic infertility. The study was supported by POS-CCE-0 2.3.1 grant 1485/SMIS-CSNR 4296.

PS14.063
DeCovA: A user-friendly tool for displaying gene coverage from Massive Parallel Sequencing data. Application to a panel of 41 genes causing epileptic disorders
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Although coverage is critical for assessing the quality and accuracy of results from massive parallel sequencing (MPS), it is often a difficult parameter to display in practice. We developed DeCovA (Depth and Coverage Analysis), a simple and easy homemade tool that combines the sequencing depth of each base and the coverage of target genes.
To illustrate DeCovA's usefulness, MPS sequencing was performed on DNA samples from 24 patients using an Ion Torrent PGM sequencer (Life Technologies). Forty-one genes that cause monogenic forms of epileptic disorders were studied in parallel.
DeCovA first determines the genomic target regions from either a bed file or a list of genes, then launches the coverageBed tool from the bedtools suite to compute the depth of sequencing on each base of these regions, for each alignment bam files to be analysed. DeCovA uses these outputs to produce graphs that show, above each gene or transcript, the stretches of DNA not covered at different thresholds set by the user. DeCovaA can also draw the sum of the samples covered at different thresholds, which is useful to identify the stretches that escape repeatedly the enrichment/sequencing methods. Bar plots can be displayed for a group of samples, allowing group or method comparisons.
DeCovA allows a fast and reliable coverage analysis. Gaps per gene are highlighted and a warning can be set in order to return correct results according to the rating level of the test (Type A, B, C of Eurogentest Guidelines).

PM14.064
Integration of Next-Generation Sequencing as a Methylation-Based Routine Diagnostic Test in Colon Cancer Tissue Screening
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Hereditary nonpolyposis colorectal cancer (HNPCC) is an autosomal inherited tumor predisposition syndrome generally caused by germline mutations in one of the mismatch repair genes (MLH1, MSH2, MSH6, PMS2) that are responsible for repairing mismatches generated during DNA synthesis. Impaired function of these MMR proteins leads to a high risk for colorectal cancer and for other HNPCC-related tumors. One of the main aim in HNPCC diagnostics is the discrimination of sporadic carcinomas from the less frequent HNPCC-associated tumors. DNA promotor methylation is typically associated with transcriptional silencing of the affected gene. Inactivation of MLH1 due to promoter hypermethylation strongly suggests a sporadic origin of the tumor. Hypermethylation of the MLH1 promoter occurs in 40% of the tumors with known negative MMR mutation status and is very rare in HNPCC patients [1]. Moreover, only methylation of CpG sites in one small region of the MLH1 promoter, the C-region, invariably correlates with silencing of MLH1 [2]. We established an assay for methylation screening of the C-region of the MLH1 promoter in colorectal cancer tissue by combining bisulfite conversion with Next Generation Sequencing (NGS). To validate this approach, we analysed the C-region of the MLH1 promoter of colorectal cancer tissue using NGS and MS-MLPA . We obtained consistent and reproducible results. NGS is a highly sensitive tool allowing an exact quantification of methylation of all CpG sites in the relevant promotor region and can easily be integrated as a methylation-based routine diagnostic test in colon cancer tissue screening.
[1] Parsons et al., Med Genet, 49(3):151-7, 2012
[2] Deng et al., Cancer Res, 59(9):2029-33, 1999

PM14.066
A systematic approach to mitochondrial DNA diagnostics through sequencing
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Mitochondrial DNA is widely investigated for diagnostics: we recently developed a pipeline to help switching mtDNA sequencing at our Institute from Sanger to next-generation techniques.
Our pipeline addresses three different mtDNA sources, genomic (with an approximate coverage of 10,000X), exome (50-200X) and long-range PCR

(~10,000X). Reads are aligned with BWA to the revised Cambridge Reference Sequence, now included in hg38 genome release. Using a linear reference for a plasmid may cause border effects, which are removed by building a secondary sequence overlapping the junction, aligning on it and merging the results at a latter stage. Nuclear DNA contamination is stripped by adding the genomic reference in the alignment step. Variant calling is performed using GATK and Pindel, while we used for the annotation step Mitomap (for known polymorphisms and known associations of mutations with diseases and somatic effects on tissues), Phylotree (for gene definitions plus tRNA definitions from the University of Leipzig), GenBank (for haplogroup definition and frequencies).
The pipeline needs approximately 15 minutes to analyze a sample, performing SNV and indel calling, heteroplasmy detection, functional annotation, haplogroup estimation, and disease annotation. Variants are stored into a database and are accessible through a Web front-end.
We created a tool focusing on diagnostics, which allows to analyze mtDNA sequences from different NGS sources, to identify disease causing variants and to provide data-quality feedback to the wet lab. The variant database will also support future phylogenetic and population based studies.

PS14.067
Roadblocks to reaching a final diagnosis with next-generation sequencing

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When does a research finding become a diagnosis? Having sufficient evidence to convert a potentially-relevant Next-Generation sequencing (NGS) finding to a concrete molecular diagnosis can be a lengthy process. This is particularly true for potentially novel disorders affecting just one family. Through our NGS-based research, we encountered a number of issues that complicated diagnosis including (i) important DNA samples unavailable for segregation analysis, (ii) only partial clinical overlap with a newly-described syndrome and (iii) novel candidate genes which require extensive functional studies. In the face of these challenges, we have had to use alternative genetic methods to determine pathogenicity.
In two studies, one X-linked recessive and one autosomal dominant, paternal DNA was not available to confirm whether the identified candidate mutations were de novo (likely pathogenic) or paternally-inherited (likely benign). Sanger sequencing and SNP haplotype analysis of unaffected siblings was necessary to determine pathogenicity. For X-linked recessive disorders, showing that the mutation is not present in any of the sisters of the patient/carrier mother can help support pathogenicity. For autosomal dominant disorders, showing that the haplotype on which the mutation is present in the patient is shared by an unaffected sibling who does not carry the mutation adds weight to the likelihood that it's de novo. For some consanguineous families we have identified causal compound heterozygous variants highlighting that we cannot always assume homozygosity despite consanguinity.
NGS has eased the identification of candidate variants. However, downstream analyses are vital to generate sufficient evidence to support causality and ensure families receive the correct molecular diagnosis.
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PM14.068
Detection of mosaicism by augmented exome sequencing

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The importance of mosaicism in Mendelian disorders is being increasingly recognized. Since many proteins form multi-unit homo-oligomers (reportedly over half), a small percentage of abnormal copies can affect a much larger fraction of these assemblies. Thus variants in less than 10% of cells can lead to serious clinical consequences. For some conditions, mosaicism explains the previously low yield of clinical genetic testing. Other conditions are obligate mosaic disorders. Furthermore, low-level parental mosaicism is increasingly recognized underlying apparently *de novo* cases of disease. Mosaicism is often undetectable through Sanger sequencing and so has been underappreciated in clinical genetic testing.
Detection of mosaicism in whole genome and exome sequencing is hampered by multiple factors. Variant calling and filtration strategies often miss or exclude variants at mosaic allele percentages. False positives can be rampant, especially over paralogous sequence regions. Read depths (typically ~30X) achieved in whole genome sequencing, add to the challenge. Exome sequencing can be much deeper, but standard exome coverage is variable, incomplete within genes, and excludes medically interpretable content outside the coding regions.
In order to detect clinically relevant mosaicism, sequencing coverage must be consistently deep over all medically interpretable content, and variant-calling algorithms appropriately adjusted. We present cases of both somatic and germline mosaicism demonstrating that the use of augmented exome sequencing with modification for mosaic detection allows for the sensitive detection of mosaicism. We demonstrate that this approach has proven more sensitive to mosaic detection than both clinical testing and some whole genome-based approaches.

PS14.069
Efficiency of a NGS panel for diagnosis of Nucleotide Excision Repair defects, and identification of a POLH founder XP mutation in Northern Spain

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Introduction: Compromised nucleotide excision repair (NER) activity causes a variety of autosomal recessive diseases including the skin-cancer predisposition disorder, xeroderma pigmentosum (XP) and the severe premature ageing condition, Cockayne syndrome (CS). Considering the clinical overlap of NER-related disorders and the several genes involved, we have developed a new diagnostic approach based on the enrichment of 16 NER-related genes by multiplex amplification coupled with next-generation sequencing (NGS).
Materials and Methods: The strategy was first evaluated on a validation cohort of 11 known patients and then applied to a prospective cohort of 30 patients. Multiplex amplification and sequencing were performed using AmpliSeq protocol on Ion Torrent PGM (Life Technologies).
Results: We detected clearly pathogenic biallelic mutations in 14 out of 30 cases (47%). Five new mutations were described in both ERCC6/CSB and ERCC8/CSA Cockayne genes. We also identified two XP patients linked to the very rarely involved ERCC3/XPB gene (only 9 patients from 6 families already published). Finally, the study of a small cohort of 4 unrelated XP patients from the Basque country (Northern Spain) retrieved a common splicing mutation in POLH (XP-variant), demonstrating a new founder effect in this population.
Conclusions: Despite the small number of NER-defective patients, NGS strategy has shown to be particularly relevant, especially for patients with atypical or incomplete clinical phenotypes. NGS is an efficient alternative to sequential Sanger approach and is the essential molecular complement of cellular tests measuring DNA repair activity.
This study was supported by a grant from Agence de Biomedecine.

PM14.070
Development of a Target Region Capture Paralleled Next Generation Sequencing Platform on Monogenic Disorders of the Newborn

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It was estimated that one quarter of the patients in the new-borns and paediatric population are actually related to genetic disorders. In new-borns whose phenotypic evidence might not be clinically manifest in early days, a definitive diagnosis may be difficult to be provided for timely treatment or prevention. Comparing with traditional methods, NGS based second-tier testing has the potential to assist in the diagnosis and management of newborn diseases by accelerating the process and reducing false positives.
A customized genetic panel was designed including 88 genes and a couple of special regions related to 51 new-born disorders. To facilitate the clinical application, we have optimized the pipeline in terms of accelerated turnaround time to ~70 hours and easy-assessed sample as dried blood spots.

The pipeline is automated to generate variation calls for SNP, Indel and large size deletions. And an in-house validated variation database was introduced to assist the interpretation of mutations.
In the assay, we first assess the performance of the pipeline using reference materials. The coverage of the interested regions is above 95% at 30X. Then 53 previously identified specimens with 65 different mutations were applied to validate the detection ability. All mutations were correctly identified except one large transposal insertion. And among 33 patients with clinically suspected symptoms, 22 patients were identified with known or suspected mutations. We thereby demonstrated that the platform is reliable to detect causal mutations and might help facilitate clinical evaluation and early intervention in newborn screening or in NICU.

PS14.071
Implementation of an NGS-based workflow for BRCA1 and BRCA2 mutation screening

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Positivity for BRCA1 and BRCA2 mutations was associated with increased probability to develop familiar breast cancer. To detect BRCA1/2 germline mutations we developed a next-generation sequencing (NGS) routine diagnostic workflow, based on the Ion Torrent PGM™ System platform. The Ion AmpliSeq™ BRCA1 and BRCA2 Community Panel was handled with a semi-automatized procedure for multiplex PCR-based library preparation and sequencing. Data analysis required the implementation of a custom designed bioinformatic pipeline for sequences alignment and for the identification, annotation and filtration of genetic variants. Sanger sequencing was performed to validate candidate mutations, and to re-sequence amplicons having low NGS coverage (<50 reads per amplicon). Negative samples were analyzed using the BRCA HP Kit (Multiplicom) for an effective homopolymeric stretches detection. This workflow together with the potentiality of our bioinformatic pipeline was blindly tested and validated onto a small cohort of patients previously Sanger sequenced, fine-tuning the parameter settings and resulting in a sensitivity of 100% in variant detection. Subsequently, 244 patients were analyzed thus confirming the need of a double check for the homopolymeric stretches with both NGS sequencing and BRCA HP Kit.
The NGS-based workflow here proposed was able to decrease the overall cost of the conventional genetic test and make the diagnostic process faster than compared to Sanger sequencing alone.
Grant references: Ministero della Salute, Ricerca Finalizzata 2009 (E35J11000190001).

PM14.072
Targeted Locus Amplification for hypothesis neutral and complete Next Generation Sequencing and haplotyping of genes of interest.

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Current methodologies in genetic diagnostics and research are limited in their ability to uncover all genetic variation in genes of interest. Clinical genetic tests often only focus on exons and therefore miss variants in the non-coding regulatory sequences of genes. In addition, structural variants, i.e. deletions/duplications (CNVs), translocations, insertions and inversions, are difficult to uncover. Their robust detection is hampered by the hypothesis-driven nature of current targeted sequencing methodologies: the collection of probes (in hybridization-based capture methods) or primers (in polymerase or ligase-based re-sequencing approaches) determines the sequences that will be analyzed. None of these methods provide haplotyping information, ultimately needed to get complete sequence information. Here we present targeted locus amplification (TLA), a strategy to selectively amplify and sequence entire genes. TLA is based on crosslinking, fragmenting and religation steps such as performed in chromatin capture technologies. We show that, unlike other targeted re-sequencing methods, TLA works without detailed prior locus information as one or a few TLA primer pairs are sufficient to amplify and sequence tens to hundreds of kilobases of surrounding sequences. This, we demonstrate, enables robust detection of single nucleotide variants, structural variants and gene fusions in clinically relevant genes. Data will be presented showing the ability of the TLA Technology to: 1) Sequence & haplotype a.o. the complete BRCA1 & BRCA2 genes for the detection of germline and somatic variation in (xenograft) tumor samples. 2) Haplotype the Human Leukocyte Antigen (HLA) region. 3) Detect all gene-fusions in genes of interest and characterize fusions at breakpoint resolution in leukemia and other cancer types.

PS14.073
Strategy for validating the ever-changing face of next generations sequencing

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Next Generation Sequencing (NGS) has seen a revolution in the last few years with the price of sequencing constantly decreasing whilst sequencing platform throughput and the range of experimental assays that can be performed, has increased. This is the result of a constant stream of NGS improvements in the form of new sequencers, new chemistry, improved protocols and new or improved software. This constant flux puts a large burden on laboratories performing clinical grade NGS, working to accreditation standards, and having to constantly re-validate these aspects before changes are implemented.
We describe the process we have introduced to clinically validate our NGS assays that range from stratified HIV sequencing to clinical exome sequencing, and encompasses all aspects from DNA sample to final report. Furthermore we discuss our long term strategy for verifying new NGS equipment and chemistry, and changes to protocol and software, to ensure they remain fit for purpose. Finally we outline and show the output of software we have created for quickly re-validating these changes at minimal cost and requiring minimal additional laboratory work.

PM14.074
A standardized validation workflow for clinical Next Generation Sequencing (NGS) analysis

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The emerging use of NGS approaches in clinical laboratories has rushed the development of guidelines to ensure adherence of the same rigorous standards as in other clinical tests for direct patient care. However, a gap remains between the general published requirements and the detailed information regarding how these should be satisfied in routine practice. Hence, we developed a standardized validation process which can be applied to NGS workflows designed to detect sequence variation within a targeted set of genes. We defined key parameters and quality criteria to assess performance and limitations within NGS-processes (platform, pipeline, test). Platform precision was evaluated by testing reproducibility and repeatability of three independent samples during the laboratory process (DNA quality, fragmentation, library/pool quantification, sequencing quality, error rate). A correlation of >95% was defined as satisfying. Pipeline accuracy was assessed through analytic sensitivity and specificity of variant calling. Two HapMap specimens were sequenced and variants detected by our bioinformatics pipeline were compared to variation data of these specimens accessible via the 1000Genomes Project. Discordant calls were confirmed by Sanger. Moreover, variants called at low allele fractions, base quality (Q<30) and coverage (<30X) were also validated to assess the limits of detection. Test validation includes mapping statistics (raw, mapped, duplicate reads) and calculation of coverage distribution (>30X) to determine the analyzable region of each clinical target. Further, evaluation of repetitive sequences, pseudogenes, homologous regions and GC content to state which parts of the clinical target may not be sequenced reliably. This validation process was designed to meet the currently published guidelines.

PS14.075
Multi-Pronged Approach for Somatic Variant Detection using HaloPlex^{HS} with Molecular Barcodes

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Agilent's HaloPlex^{HS} is a next generation PCR target enrichment method that enables enrichment of thousands of targets in a single tube. The protocol utilizes specificity gained from restriction enzyme digestion, hybridization and DNA ligation to capture the target region. Standard amplicon-based target enrichment methods are limited in sensitivity because mapping start points of paired end reads cannot be used to identify unique molecules and improve confidence in variant calling. Therefore, we have added molecular barcodes to the primer cassettes allowing detection of unique molecules.
The HaloPlex^{HS} workflow has been optimized to take <6 hours to complete and requires only 50 ng input. Using molecular barcodes, we demonstrate increased confidence and detection of variants down to <1% allelic fraction.

Furthermore, an improved probe design in Agilent's SureDesign results in increased coverage for custom designs. We demonstrate for 31 designs, >85% specificity and >90% of target regions covered at 10% of average depth when sequenced at >100X.
Finally, Agilent's SureCall 3.0 utilizes the molecular barcodes in a novel algorithm for removing duplicate PCR fragments. It assigns reads to amplicons by probe sequence matching, then uses the barcodes to merge duplicate reads while providing correction for sequencing and PCR errors and improving variant calling. SureCall can accurately call variants at 2.5% allelic fraction. We demonstrate the speed and accuracy of this method, especially for reads with non-unique mapping. With this multi-pronged approach, from probe design, to library prep, to analysis, we demonstrate that HaloPlex^{HS} provides more accurate variant calls with higher sensitivity and better coverage of custom designs.

PM14.076

The comprehensive analysis of a cohort of Polish patients with the suspicion of neurofibromatosis type 1 using Next Generation Sequencing

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Neurofibromatosis type 1 (NF1) is a common autosomal dominant disorder affecting approximately 1 in 2000 to 3500 people. It appears to be a single gene-determined disorder characterized by complex cognitive symptoms, including cutaneous neurofibromas, cafe-au-lait spots, Lisch nodules and optic gliomas. The *NF1* gene has one of the highest rates of mutations in the human genome, with almost equal split between spontaneous and inherited mutations. Identifying the disease-causing change is complex due to the large size of gene, the presence of pseudogenes and the lack of mutation hotspots.
The present study describes screening for the *NF1* mutations in 90 patients from 76 unrelated families with phenotype fulfilling diagnostic criteria or with suspicion of neurofibromatosis type 1. The analysis included amplification of 58 coding regions and flanking splice sites (according to NM_001042492.2), library preparation and next-generation sequencing (NGS). Sanger sequencing was used for mutation confirmation or when the average coverage per region was lower than 50x. To the date of submission, 49 unique mutations were identified within 76 families, including 7 missense substitutions, 16 stopgain mutations, 19 small deletions or insertions and 7 splice site changes. Twenty one out of these mutations are registered in the Human Gene Mutation Database, four variations concern the location where a different mutation has been previously discovered and the rest are newly detected mutations with a probable pathogenicity.
This is the first comprehensive analysis of the *NF1* mutational spectrum in Polish patients with the neurofibromatosis type 1 suspicion.
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PS14.077

New methodology for NGS analysis of Dushenne Muscular Dystrophy.

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Next-generation sequencing (NGS) is a new group of methods, which widely used in DNA-diagnostics lately. Whole genome sequencing, exome sequencing and different panels of causes genes are applied in clinical medicine. Advantages include: relative cost-effectiveness, analysis all potential genes at once and rapidity of diagnostic. But these methods still have problems like low/no coverage some regions and no information about it, too much information and difficulty of it processing, absence certificate for medicine.
This work purpose was development of methods Dushenne Muscular Dystrophy (DMD) diagnostic by NGS, which can use in medical practice.
The basis of our methods was AmpliSeq technology but we added one stage more. We designed own primers for all exons and adjacent intron regions of DMD, EMD, FHL and LMNA A/C genes which had used at the first stage. After PCR we have multiplex of 105 amplicons. Then we had made fragmentary analysis after second PCR with fluorescently labeled universal primer. Therefore, it becomes possible to assess quality and quantity of each amplicon. This added stage saved us the trouble of low/no or different coverage between some regions. Then we had been convinced that all fragments were in the same concentration we made PCR with sequencing primers.
Ten DMD patients have been analyzed by the claimed method on Junior (Rosh) equipment. All exons were analyzed, coverage different was 7x. Three of patients had mutation in DMD gene. Because we sequenced limited part of genome, it had no difficulty to processing NGS results.

PM14.078

NGS sounds really good! But, what kind of NGS-based test might I offer to my patient?

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Introduction: NGS is being established as a new alternative for the diagnosis of genetic diseases in the clinical practice. The great advantages of this promising technology have been duly demonstrated in research. But some considerations need to be taken into account before being implemented as a clinical routine. NGS is a great technology, or better said, a set of technologies and different applications, but more important than the technology itself is the use that is made of it.
Material and Methods: We have developed different NGS based strategies for the analysis of genetic conditions. An accurate and highly sensitive approach based on a PCR specific design, which we have called NextGeneDx®. This approach was extensively validated to be used for diagnostic applications. An exome analysis focused only to the genes associated to clinical phenotypes according to OMIM (Clinical Exome). And a targeted or Ad Hoc Exome focused to the genes associated to a specific condition or phenotype.
Sspecific bioinformatics pipelines and interpretation algorithms were implemented for all these strategies.
Conclusions: NextGeneDx® is a diagnostic and accurate NGS alternative to Sanger for genetically heterogeneous diseases or phenotypes associated to a limited number of genes. Ad Hoc Exome provides a high sensitivity and specificity and allows the exhaustive analysis of a high number of genes associated to a specific condition. Clinical exome provides the analysis of all the genes associated to a OMIM phenotype making easier the clinical interpretation of the results. This approach is very useful for unspecific conditions or phenotypes.

PS14.079

NextGeneDX®: An accurate and cost-effective NGS Application for genetic diagnosis

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NGS is a very promising technology that allows to analyse millions of sequences by using a single assay, reducing costs and time significantly compared to Sanger sequencing. However, the use of NGS in routine clinical practice is still under discussion because, inter alia, variations in the specificity and sensibility associated, mainly, to the capture systems. NextGeneDX® is a NGS-based procedure validated for diagnostic applications in the clinical practice. This PCR-based targeted capture system has been developed, validated and implemented for a wide range of genetic diagnosis in the last two years.
Last years, 233 genes were analysed in 1670 patients with phenotypes associated to 73 different genetic diseases.
Capture of the regions of interest (ROIs) has been performed by specific PCR. Specificity and analytical accuracy of the PCR design have been checked by Sanger analysis. Libraries were prepared according to NexteraXT protocol and sequenced on a MiSeq. The analysis and interpretation of the results have been performed according to our own algorithms.
All pathological or probably pathological changes were confirmed by Sanger sequencing.
Our NGS-based procedure for diagnosis allowed the identification of the disease-causing change in 403 patients (24%). To date, false positives have not been detected using NextGeneDX® strategy.
NextGeneDx® approach has the same sensitivity and specificity than Sanger sequencing, with a 100% of representativity of the ROIs and a minimum depth of 100X. It is a diagnostic alternative to Sanger sequencing for analyzing large genes or several genes, associated to multigenic or genetically heterogeneous diseases, simultaneously reducing, significantly, times and costs without compromising the diagnostic accuracy and quality.

PM14.080

Next-generation errors in medical practice: A topic for clinical and laboratory geneticists to address in collaboration.

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The introduction of next generation sequencing (NGS) techniques,although generally meeting its high expectations, causes new problems in clinical genetics. When testing a single gene, the prior probability of causality is high for suspicious variants, which led to the erroneous perception that any abnormal DNA test result is 100% specific. The prior probability of causality is dramatically lower when large gene panels or whole exome sequencing (WES) is performed. This demands a sceptic attitude and a higher burden of proof for causality. Rigorous assessment of the genotype-phenotype relation (deep phenotyping) is essential, and should be jointly assessed with the pathogenicity of the variant on molecular basis alone.
We have collected a series of cases illustrating these issues and have extracted several apparently common causes for mistakes:
A.
Genotype interpretation mistakes: (1) unfamiliarity with a gene; (2) over-interpretation of variant significance; (3) bio-informatic mistakes (4) misjudging what is detectable with NGS.
B.
Fenotype interpretation mistakes: (1) superficial phenotyping; (2) suboptimal utilization of phenotypic data;
Based on these errors from practice, we recommend that (I) Laboratory and clinical geneticists share responsibility for final test results; (II) DNA laboratories remain specialized on (groups of) genes; (III) Large gene panel analyses and WES should only be requested in collaboration with clinical geneticists.
We recognize that these recommendations need refinement. However, the misconception that NGS is the panacea of genetic diagnostics should be challenged, to prevent possibly serious errors. Close collaboration between clinical and laboratory specialists to optimize patient care and safety is needed.

PS14.081

Exome-wide SNP genotyping as a tool for validation of a large diagnostic next-generation sequencing panel

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Validating the sensitivity of next-generation sequencing (NGS) for diagnostic testing is essential. This involves confirming the detection of a number of positive control variants (60 to confirm >95% sensitivity, 300 for >99% sensitivity at the 95% confidence interval (CI)). Samples with known variants from previous Sanger sequencing tests can be resequenced, though this can prove costly, necessitating the NGS of many samples. We present the validation of a Neurogenetics-specific Illumina Nextera Rapid Capture Custom NGS panel of 153 genes using the Illumina Human Infinium Exome-wide BeadChip array v1.2, which includes over 240,000 exonic variants. Ten control samples were SNP-genotyped (one in triplicate, giving identical calls between the replicates). A total of 134 unique single-nucleotide variations were detected in the targeted genes, with a grand total of 311 heterozygous and 152 homozygous. All were correctly identified by NGS. There were discordant genotype calls for 2 variants, Sanger sequencing verified that the NGS was correct for both. Six samples with indels from 1-40 bp in length were also tested, all were detected. This is a total of 140 unique variants, from which a sensitivity of >97.9% at the 95% CI can be derived. Our validation also included the repeat sequencing of two samples on different runs and downsampling to establish the lowest read-depth required to call a variant. The 10 SNP-genotyped samples will provide a valuable resource for periodic verification of the performance of our panel, and validation of future NGS tests.

PM14.082

Optimization of Library Amplification For Next Generation Sequencing

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Uniform coverage of all genomic regions during Next Generation Sequencing (NGS) is critical for efficiently utilizing sequencing capacity and preventing loss of important sequence information due to drop-out or under-representation of certain regions. The coverage uniformity is especially important in applications such as microbiome-sequencing, where different microbial strains could have significantly different GC contents. GC content -related sequencing bias could potentially lead to under-representation or even complete loss of the genomic regions or microbial strains with very low or very high percentage of GC bases. The PCR step of the NGS library construction procedure has been shown to be the major source of GC bias in the NGS workflow. To solve this common problem in the NGS field, we established a test system where a mixture of high-GC and low-GC bacteria genomes is used to optimize library amplification conditions and used this system to develop a novel NGS library amplification mix that amplifies the genomes with widely different GC contents with minimal bias and high fidelity.

PS14.083

Is exome sequencing a reproducible technique under routine conditions ?

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Exome sequencing is commonly used for research or diagnosis purposes, sometimes without notion of robustness or reproducibility of the chosen techniques. However these criteria must be considered to validate NGS data. Here we present the results of an exhaustive comparison of the quality data in a series of exomes performed at the Genome Transcriptome platform of the Functional Genomics Center of Bordeaux.
Comparisons of the quality data have been performed in three steps, from raw data (FASTQ), aligned reads (BAM), and detected variants (VCF), as described in Guo and al.1. We compared 2 different libraries (SureSelect and Nextera) sequenced on a MiSeq system. In a first set, 2 exomes were performed from the same DNA sample and in a second set 4 exomes were performed from 2 different DNA extractions.
These biological replicates allowed us to evaluate the loss of information throughout the bioinformatic pipeline, because of low quality raw data, and its impact on the number of relevant variants detected. Thus, by comparing the concordance of variants detected in the 4 exomes set, we were able to show which technique of library preparation gives the most reproducible results, and which types of patient's mutations are most likely undetected by each kit.
This work allowed us to accurately assess the quality of exome datasets obtained by NGS sequencing in routine conditions, with two different library kits. This validation procedure could help in the future to make more relevant choices among the available NGS techniques.
1 Three-stage quality control strategies for DNA re-sequencing data. Guo et al. Brief Bioinform. 2014 Nov;15(6):879-89

PM14.084

Non-invasive prenatal diagnosis (NIPD) of Cystic fibrosis (CF): an optimized protocol using multiplex fluorescent PCR to detect the p.Phe508del mutation

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Analysis of cell-free fetal DNA (cffDNA) in maternal plasma is very promising for early diagnosis of monogenic diseases. However, NIPD of single-gene disorders has been limited by the availability of suitable technical platforms and the need to set up patient or disease specific custom-made approaches. To make clinical applications more readily accessible, we propose a simple test based on multiplex fluorescent PCR and size fragment analysis to determine the paternally inherited fetal allele. Cystic fibrosis (CF) has been taken as a model to evaluate this approach, in couples with high risk of transmitting the disease. This test combines the detection of p.Phe508del (the most frequent mutation in CF patients worldwide), nine intragenic and extragenic STR markers of the *CFTR* locus and a specific SRY sequence. All primers were designed regarding the size of cffDNA (i.e. to amplify short fragments).
This multiplex PCR protocol was successfully applied to 5 couples where fathers carried the p.Phe508del and mothers were carrying a different mutation. Our simple test provided clear results on the maternal plasma from the pregnant women. We confirmed the presence of cffDNA in the studied samples by the identification of a tri-allelic DNA profile using a miniSTR kit. All results were correlated with chorionic villus sampling or amniocentesis analyses.
This NIPD test, focused on both direct and indirect diagnosis of CF, offers many advantages over current methods: simple, rapid and cost-effective. It opens up possibilities to test a large number of couples with high risk of CF

for offspring.
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PS14.085

Karyotype level non-invasive prenatal testing by sequencing of circulating cell-free DNA from maternal plasma

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Non-invasive prenatal testing (NIPT) has become an accepted method for the detection of trisomy 21, 18 and 13 in patients at high risk to carry a fetus with one of these chromosomal abnormalities. NIPT can also be used to detect other aneuploidies and sub-chromosomal copy number variations. With the inevitable progress of sequencing technologies, it is likely that sequencing-based NIPT will offer greater diagnostic capabilities in the same way that karyotyping-level resolution was eventually superseded by microarray analysis of invasively acquired fetal cells or tissue. We present studies that support the concept of NIPT delivering genome-wide unbalanced copy number results, analogous to what can be detected by cytogenetic G-band karyotyping.
We analyzed a set of maternal plasma samples for which karyotype confirmation of fetal anomalies were available. Next, we constructed a comprehensive *in-silico* system of maternal plasma DNA sequencing results modeled to contain sub-chromosomal events at genomic coordinates reported in the ISCA database. Finally, we constructed an analytical model system with both karyotype and microarray confirmed samples with genome wide sub-chromosomal deletion and duplication events.
In karyotype confirmed samples, we detected 17 of 18 samples with a sub-chromosomal deletion or duplication. *In-silico* modeling of 587 genome wide ISCA sub-chromosomal deletion and duplication events ≥7 Mb showed that sensitivity could reach 94% with high specificity.
Using whole genome sequencing results, we were able to model *in-silico* genome wide events and estimate sensitivity metrics for their detection. Measurement of analytical performance in mixture models with confirmed deletions/duplications is currently ongoing.

PM14.086

A new NGS-based assay for detecting PALB2 variants in familial breast & pancreatic cancer

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Genetic testing for breast and ovarian cancer susceptibility genes, BRCA1 and BRCA2, is well established for the early identification and management of hereditary cancers. Another gene involved in the DNA damage repair (DDR) pathway is PALB2 (partner and localizer of BRCA2) or FANCN. It recruits BRCA2 to BRCA1-bound damaged DNA sites, to mediate homologous recombination. Heterozygous PALB2 mutant carriers have significant cellular DNA repair defects, whilst homozygous mutant patients develop Fanconi anemia. We recently showed that heterozygous carriers of deleterious PALB2 germline mutations have at least 5 times greater predisposition to developing breast cancer compared to the general population, with increasing risk in younger age groups. Carriers are also predisposed to pancreatic cancer, independent of BRCA1 or BRCA2 mutations.
We have developed a low-cost clinical assay for PALB2 variant detection which complements the existing BRCA1 and BRCA2 assays offered in our NHS clinical laboratory. It combines long-range PCR amplification with next generation sequencing (NGS). This provides high sequencing depth for confident variant calling and flexibility at multiplexing, thus minimizing individual sample costs. Previously known PALB2 wild-type and mutant DNA samples, detected by other NGS methods, are used to validate the assay. Another clinical utility could be for newly identified mutant PALB2 carrier breast cancer patients to be considered as candidates for PARP inhibitor therapeutics, targeting defective DDR. However, the assay will initially be included for breast and pancreas cancer screening at our NHS genetics laboratory and cost efficiency analysis is ongoing, comparing against other targeted resequencing panels.

PS14.087

Ten Years of EMQN Phenylketonuria EQA scheme

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Objective
Analysis of the last ten EMQN-EQA (European Molecular Quality Network-European Quality Assessment) schemes regarding molecular diagnosis of Phenylketonuria (PKU)
Basics
PKU (OMIM # 261600) is an autosomal recessive metabolic disorder due to mutations in the PAH gene. Deficiency of phenylalanine hydroxylase in PKU causes excess of phenylalanine which affects brain development and function. Over 600 pathogenic PAH variants are known. Depending on mutations the phenotype severity varies from mild hyperphenylalaninemia (MHP) to mild and severe PKU. Early molecular diagnosis of PKU and correct interpretation is important for treatment planning including possible tetrahydrobiopterin cofactor responsiveness.
Method
Each year this EQA scheme consisted of three simulated cases. Over the 10 years 23 different variants and 26 different genotypes were distributed for analysis. Nearly 550 mock reports were quantitatively and qualitatively analyzed for a range of criteria including genotyping, interpretation and clerical accuracy.
Result
Participants came from 23 different countries including Australia and the US; participant number increased from 11 in 2004 to 27 in 2014. The total genotyping error rate has decreased from a maximum of 20% to 1.4% although in every year at least one lab missed at least one mutation. Many laboratories have serious shortcomings in genotype-phenotype predictions. 2007 and 2012 several laboratories offered prenatal testing for genotypes not associated with clinical symptoms (MHP).
Discussion
Quality of PAH genotyping has improved over the years but mutation interpretation remains a major challenge for a number of laboratories. Availability of improved mutation databases would probably help to further improve diagnostic quality.
Grant reference: EMQN

PM14.088

Real-time quantitative PCR for copy number analysis: has our service improved?

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Real-time quantitative PCR (qPCR) to determine copy number was introduced as a diagnostic service by the All Wales Medical Genetics Service (AWMGS) in June 2014. At this time increasing demand on the laboratory budget, in particular for the send-out budget, meant that there was an urgent need to develop a cost effective strategy for validation of dosage results, particularly following array CGH testing. An in-house qPCR method was developed and validated that allowed cost effective, flexible assay design for unique and recurrent genomic regions. We are aware that only a few laboratories within the UK have adopted this testing strategy. There are no current professional guidelines or external quality assessment schemes for qPCR.
Review of the first 6 months post implementation of this service for validation of array CGH imbalances showed evidence of the cost benefit of this service compared with previously available follow up techniques. In addition, the clinical utility of this testing has also been demonstrated through detection of a homozygous imbalance in a proband who had inherited the deletion from each of his hemizygous parents. More recently, qPCR testing has also been applied for confirmation of potential dosage imbalances detected by next generation sequencing (NGS) assays within this laboratory. Further advantages following implementation include targeted prenatal testing and targeted familial studies, eliminating the possibility of detecting incidental findings.
We present a review of the first 11 months of the qPCR service to evaluate whether we have improved our laboratory services.

PS14.089

QSTR*R-PL: Development and Validation of a QF-PCR based IVD for Rapid Aneuploidy Screening in the Event of Pregnancy Loss

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Introduction: Elucigene Diagnostics has developed QST*R-PL, a simple

to use assay for the rapid and accurate diagnosis of the six most common autosomal trisomies associated with miscarriage. QST*R-PL employs the commonly used QF-PCR (Quantitative Fluorescence-PCR) technique, which offers numerous advantages over the techniques currently used in the diagnosis of miscarriage.
Methods: A QST*R-PL performance evaluation assay was provided by Elucigene Diagnostics and evaluated using protocols developed by Elucigene Diagnostics. Testing involved 150 anonymised DNA samples extracted from fetally derived tissues/product of conception, whose aneuploidy status was previously determined using alternative testing methods including Karyotyping, FISH and Microarray. QST*R-PL test accuracy was assessed through concordance with the previous diagnosis.
Results: The QST*R-PL data demonstrated high quality results across all testing sites. Comparison of the QST*R-PL diagnostic results with that of the previous diagnostic data showed 100% concordance with no failed results.
Conclusion: The QST*R-PL assay used in the clinical setting can provide users with a rapid aneuploidy screening service for miscarriage cases and can easily integrate into any existing QF-PCR based prenatal service. QST*R-PL also enables the identification of maternal cell contamination and specific cases of triploidy which are less easily detectible using current diagnostic methods. Such a service can provide closure to patients or couples who have undergone a traumatic miscarriage event and can aid the management by modification of risk calculations for future pregnancies and can change the way recurrent pregnancy loss is managed clinically in the future.

PM14.090
Whole transcriptome next-generation sequencing to detect gene fusions in haematological malignancies

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Introduction: Current methods of detection of genetic markers for haematological malignancies include the identification of gene-fusions using fluorescence *in situ* hybridisation (FISH), karyotyping, real-time PCR (RT-PCR) and screening genes for mutations through targeted sequencing. However, Next-Generation Sequencing (NGS) has a number of potential advantages over traditional methods. We aimed to establish a workflow for detecting gene-fusion transcripts using NGS, in order to develop a service to detect a range of diagnostic and prognostic genetic markers of haematological malignancies. We have validated this method through the detection of recurrent, well-characterised *BCR-ABL1* gene fusions in patients with chronic myeloid leukaemia (CML) fusions as a proof of principle study.
Method: Whole transcriptome libraries were prepared from RNA extracted from the blood and bone marrow of patients with chronic myeloid leukaemia (CML) and acute lymphoblastic leukaemia (ALL) and known *BCR/ABL1* fusions using the Illumina TruSeq RNA Access library prep kit. These libraries were sequenced on the Illumina HiSeq 2500 and a bioinformatics pipeline was developed to detect the gene fusions.
Results: This method detected the *BCR/ABL1* fusions in the patient samples and can distinguish between different breakpoints in the genes. A comparison of different algorithms for the detection of gene fusions and a cost-benefit analysis of this technique will also be presented.
Conclusion: We have shown that whole transcriptome NGS is a promising method for detecting gene-fusions which can be developed further to detect genetic markers in a range of complex haematological malignancies.

PS14.091
Development of a Next Generation Sequencing (NGS) gene panel assay for Segmental Overgrowth Syndromes.

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Segmental overgrowth syndromes (SOS) are a heterogeneous group of rare diseases characterised by substantial localised or asymmetrical excessive tissue growth manifesting at birth or later in life. SOS encompass disorders such as Megalencephaly-Capillary Malformation (MCM/MCAP) syndrome, Megalencephaly-Polymicrogyria-Polydactyly-Hydrocephalus (MPPH) syndrome, Congenital Lipomatous Overgrowth Vascular Malformations, Epidermal Nevi and Skeletal abnormalities (CLOVES syndrome), Proteus syndrome and Cowden syndrome. Due to overlapping phenotypes clinical diagnosis can be challenging.
Many SOS have been attributed to mutations in genes of the phosphoinositide 3-kinase PI3K-Akt signalling pathway, with both germline and somatic mutations associated. We have developed a Next Generation Sequencing (NGS) assay for key genes in this pathway with whole gene screening of PIK3CA and PTEN, and targeted screening of hotspot exons in PIK3R2, AKT1, AKT3, mTOR and CCND2. This assay uses long range-PCR as the target enrichment strategy and Illumina's MiSeq system to perform next generation sequencing, followed by analysis using a custom bioinformatics pipeline.
Assay validation was undertaken across five independent MiSeq runs comprising 20 analyses of 12 patient samples previously screened by pyrosequencing or Sanger sequencing. NGS data was directly compared to previous data with fully concordant results. Assay repeatability and sensitivity were determined, and the bioinformatics pipeline was validated to detect mutations down to a 5% admixture.
As a result of this validation, the NGS Segmental Overgrowth Syndrome assay is now offered at the Manchester Centre for Genomic Medicine. Potentially disease causing mutations have been identified in 50% of patients tested so far and confirmed using Sanger sequencing or ARMS-PCR.

PM14.092
European Molecular Genetics Quality Network (EMQN): First experience with External Quality Assessment for the molecular diagnosis of SHOX-deficiency in Europe

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The European Molecular Genetics Quality Network (EMQN) is a not-for-profit organization promoting quality in genetic testing worldwide by provision of standard External Quality Assessment (EQA) schemes and agreed best practice. Since 2011, SHOX-deficiency has been one of these EQA schemes and involves sending three DNA samples, with matched mock clinical referrals, for analysis by the participating laboratories. A panel of three assessors using previously agreed marking criteria anonymously marks the resulting clinical reports. Each participating laboratory is scored for genotyping, interpretation and clerical accuracy (max score is 2.00 per category), and tailored comments are fed back. A report summarizing the results of the scheme is also provided.
In 2014, a total of 43 laboratories from 15 countries participated in the SHOX scheme. One case involving a patient with disproportionate short stature and Madelung deformity (SHOX p.Ala170Pro mutation), had a worryingly high genotyping error rate of 26%. This mutation has previously been reported to cause an aberrant signal for the exon 4 MLPA probe of the most commonly used MLPA kit. Many laboratories did not follow best practice and failed to use an alternative method to verify this abnormal finding leading to an incorrect result. Consequently, the mean genotyping error rate for the scheme was high (8.77%). A full summary of all the results of the SHOX schemes run between 2011-2014 will be presented. The errors identified indicate a clear need for EQA to improve the standards of technical and reporting performance in clinical diagnostic laboratories offering a SHOX testing service.

PS14.093
Efficient, targeted personalized medicine by BRAF, KRAS and NRAS genotyping on a benchtop sequencer

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Reliable tumor tissue molecular analysis with high sensitivity and specificity is crucial to guide correct personalized medicine in cancer. Somatic mutation detection in several codons of the BRAF, KRAS and NRAS genes has a great impact on colorectal cancer treatment decision. The release of benchtop Next Generation Sequencing (NGS) instruments has paved the way to implement the NGS chemistry in a clinical setting without making sacrifices in terms of cost and turnaround time.
We developed and validated a PCR based NGS approach for mutation detection in Formalin Fixed Paraffin Embedded (FFPE) samples. Nine BRAF, KRAS and NRAS target codons are amplified in seven reactions followed by NexteraXT library preparation and sequencing on a Miseq. Except for the challenge of performing NGS with FFPE samples in terms of DNA quality and homogeneity, a thorough validation of the detection limit appeared to be essential. The detection limit is influenced by different factors including the quality of the specimen, the sequence to be analyzed, primer design and PCR bias. We investigated these factors resulting in a reliable detection of the variants down to 3% with a minimal coverage of 1000x of each target. Hence,

our approach can identify the selected mutations with a sensitivity unseen with traditional Sanger sequencing and directly quantifies the amount of the mutant allele. In brief, we developed a simple, robust, flexible and sensitive sequencing assay for somatic mutation detection in colorectal cancer using NGS, which can serve as a model for tumor tissue molecular analysis for other acquired diseases.

PM14.094
Ten years of SCA external quality assessment (EQA) schemes organised by EMQN

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Spinocerebellar ataxias (SCAs) are autosomal dominant neurodegenerative diseases, caused by triplet-repeat expansions in the most common forms. An EQA scheme, provided by EMQN, has been running since 2004. We assessed reports for 3 mock-cases, every year, for genotype and interpretation (score: 0-2). Laboratories were expected to discriminate, and accurately size, both allele repeats for SCA1, SCA2, MJD/SCA3, SCA6 and SCA7; assessment included also (1) pre-test requirements; (2) appropriate methodologies, updated knowledge and acceptable repeat range; and (3) interpretation, reporting and recommendations.
Laboratories (28-73; mean: 51) from 34 countries (mean: 22), from all continents, increased over time. We found an improvement in genotyping and interpretation, and a decrease of gross errors (range: 0-5%). Mean scores were compared according to the participation of each lab (once, 2-6, 7-10 times). Differences (factorial ANOVA) were not significant, but labs that participated only once had lower scores (1.75) and the greatest heterogeneity. Labs participating frequently had higher scores (1.89) and the most consistent results.
Many labs do not treat EQA as their routine diagnostics. Other common problems included: missing homoallelism; not specifying both allele sizes; not reporting size of normal and/or expanded alleles; out of acceptable margins of error; not offering the minimum "menu" for SCA testing.
EMQN best practice guidelines were published in 2010, to help improving the quality of SCA testing. This EQA scheme shows a global progress in laboratory SCA testing performance. Labs participating more often showed an overall better performance. These results emphasize the importance of regular participation in EQA schemes.

PS14.095
Mapping the "Dark Matter" of Genome using Nanochannels - Long repeats, Complex Structural Variations and Their Biological Relevance

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Despite NGS advancements, portions of the human genome remain unresolved. During assembly, gaps and unknown structural information, the "dark matter" of the genome, is challenging to detect with current technologies. Rapid comprehensive genome mapping in NanoChannel Arrays represents a complementary platform to DNA sequencing. *De novo* assembly of single molecules yields unprecedented long contiguous maps, advantageous in highly repetitive regions and complex structures in their native form.
We present results showing hundreds of large structural variants and haplotype differences in genomes; 11% of 24,360 large SVs found in the 22 euploid human genome are unique to a specific genome while 23% of those SVs were common to 20/22 samples. In 1 human, we detected >700 of insertions/deletions and inversions >1 kb. Without considering SVs that overlap with N-base gaps in hg19, 90% of these SVs are supported by orthogonal experimental methods or historical evidence. A high portion of complex genomes is composed of previously unknown repeating units (>2 kb) spanning several tens of kilobases to megabases, the exact locations and copy numbers often remain elusive with NGS. Without knowing the genomic context or amount of these repeats, it is difficult to attach biological relevance to them. Using BioNano's Irys® platform, repeat regions can be more accurately characterized and put into context. We found repeats and complex SV regions spanning 100-200+ kb that are clinically associated with diseases or disorders. For the first time, population scale cross-sample genome comparison to identify comprehensive genomic structural variation is feasible on a single platform.

PM14.096
Evaluation of SureSelectQXT Target Enrichment library preparation for Illumina parallel sequencing

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Parallel sequencing library preparation protocols using minimal sample input and manipulation, while maintaining high sensitivity are an essential requirement for a clinical laboratory. Agilent Technologies SureSelectQXT kit improves on their standard SureSelectXT library preparation times with as little as 50ng of input DNA. Using a transposase-based library preparation with improved hybridization that decreases both hands-on-time and hybridization time from 16 hours to 90 minutes.
We have evaluated the kit using 4 different focused target-enrichment gene panels (Congenital Myopothies; Congenital Muscular Dystrophies, In-born Metabolic Diseases and Breast Cancer) and 41 samples comprising of 44 previously identified variants (28 SNVs and 16 CNVs). Most of these variants were previously sequenced using the SureSelectXT protocol with Covaris randon-shearing and although the SureSelectQXT shearing is not entirely random, all the expected variants were detected. Additionally CNV coverage data from the QXT protocol seems to show more consistent and less variable CNV data compared to the Covaris SureSelectXT protocol.
Sequencing results show both a comparable percentage of on-target sequencing reads and a high concordance of variants detected by the SureSelectQXT protocol and known previously identified variants in the these samples. Overall the SureSelectQXT library preparation kit showed confident coverage of the genomic regions of interest tested here, allowing us to perform both point mutation analysis and CNV analysis from the same data. Furthermore this protocol has a reduced number of steps providing a streamlined approach that is amenable to automation on an Agilent Bravo workstation, allowing us dramatically increase our throughput from 96 to 288 samples a week.

PS14.097
A novel method for DNA and RNA target enrichment

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Recent developments in next-generation sequencing (NGS) technologies have made a great impact on medical research. However, in order for NGS to be fully incorporated into routine diagnostic genetic testing, target enrichment methods are required that are fast, simple, and cost effective. To meet these requirements, we have developed a novel target capture technique that offers significant advantages over traditional in-solution hybridization and multiplex PCR protocols.
Our method utilizes rapid enrichment of target DNA or cDNA through the hybridization of short probes to fragmented DNA. The probes both enable the capture of the target DNA to beads and define the boundaries of the regions of interests. Off-target sequence is removed by enzymatic digestion, followed by ligation of platform-specific adaptors to the trimmed targets and PCR amplification.
To demonstrate the high specificity, sensitivity and uniformity of this method, we have applied it to the capture of genes commonly mutated in cancer.

PM14.098
GenoDENT, a targeted next-generation sequencing assay for the diagnosis and discovery of mutations in orodental disorders

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Oro-dental genetic disorders can manifest in both syndromic and non-syndromic forms. Of the >5000 syndromes listed in the London Dysmorphology Database, >900 have been documented to have dental manifestations. Therefore, the dental consultation can be an important port of entry for the management of a number of genetic disorders. We have developed a targeted next-generation sequencing (NGS) assay, GenoDENT, for the diagnosis and discovery of mutations in 560 known and candidate genes in orodental diseases. We demonstrate the utility of this assay for the molecular diagnosis of a variety of syndromic and non-syndromic dental disorders, eg. amelogenesis imperfecta, selective tooth agenesis, ectodermal dysplasias etc. In a cohort of 104 patients referred to the Reference Centre for Orodental Manifestations, Strasbourg, France, we delivered a definitive molecular diagnosis in 38 cases (~37%). Interestingly, in several cases a molecular diagnosis for the dental disorder led to a refinement of the patient's phenotype and supplementary clinical tests in cases suggestive of syndromes, thus having a direct impact on overall patient care. GenoDENT provides superior coverage for the targeted region as compared to whole exome sequencing (Mean Coverage: GenoDENT - 371X vs. Exome V5 - 96X; GenoDENT - 98% nucleotides covered at >20X vs. Exome V5 - 87% nucleotides covered at >20X) at lower cost. Therefore, we have developed the first NGS assay to target genes implicated in orodental disorders and development. This EU-funded project (ERDF) A27 „Oro-dental manifestations of rare diseases", is supported by the RMT-TMO Offensive Sciences initiative, INTERREG IV Upper Rhine program www.genosmile.eu.

PS14.099

High efficiency targeted sequencing for accurate identification of low frequency somatic variation in cancer

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Somatic genome variation is a key feature underlying the pronounced cellular and clinical heterogeneity observed across a wide range of cancers. The ability to detect and accurately quantify this variation in tumors, particularly as it evolves over time, will be useful for predicting and monitoring the effectiveness of therapy, the emergence of pharmacoresistance and metastasis, and projecting a patient's prognosis. An effective solution for this challenging application must be sensitive, accurate, reproducible, fast, low-cost and automatable, with robust data analysis and reporting outputs. We developed a targeted NGS method called HEAT-Seq (High Efficiency Amplification of Targets for Sequencing) based on highly optimized, multiplexed, molecular inversion probes. HEAT-Seq probes incorporate unique molecule identifiers (UIDs) to facilitate the tagging of PCR duplicates and accurate assessment of molecular complexity free of amplification bias. We evaluated the performance of this technology using a series of normal and cancer reference samples. A panel of ~700 HEAT-Seq probes targeting mutational hot spots within a set of 55 cancer genes, coupled with Illumina MiSeq sequencing, demonstrated that >98% of the target region was covered to at least 50x coverage depth, with a duplicate read rate less than 20%, and with >96% of the probes exhibiting ≥20% of the mean panel coverage. Initial evaluation of sensitivity for this panel demonstrated that known single nucleotide variants in the samples could be reliably detected when present at frequencies down to below 1%. We conclude that optimized HEAT-Seq panels are a promising solution for important applications in cancer genomics.

PM14.100

The new Illumina TruSeq Exome Enrichment kit optimized for less oxidative damage, higher enrichment efficiency and higher uniformity of coverage.

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Starting from only 100ng of gDNA the newly developed Illumina TruSeq Exome Enrichment kit delivers comprehensive and highly uniform coverage of exon sequences. The product workflow consists of three general steps: acoustic DNA shearing, library preparation and rapid capture exon enrichment step. All steps have been optimized to provide high percentage of reads on target, high coverage uniformity and minimized oxidative DNA damage.
It was demonstrated that mutations can be introduced during acoustic shearing. To counter the observed oxidative damage we added EDTA to our shearing buffer. Furthermore, we optimized the library preparation procedure to obtain median insert size of ~150bp, which provides optimal enrichment metrics. Also, we reduced the number of PCR cycles to limit the amount of duplicates introduced during amplification, but still obtain sufficient yield to enable flexibility in choosing the amount sample input into enrichment.
The TruSeq Exome Enrichment kit is designed for use with Illumina's Coding, Expanded and Custom Exome pools selected using Illumina's DesignStudio. During enrichment optimization we validated a new wash buffer that further protects the DNA from artificially induced mutations. The combination of EDTA in the shearing buffer and the new wash buffer allows us to decrease probability of incorrect variant calls.
We have enabled pre-enrichment pooling of up to 12 libraries for higher throughput without compromising standard enrichment metrics of >80% reads on-target. Both the high enrichment efficiency and high coverage uniformity makes our new TruSeq Exome Enrichment kit an ideal tool for analyzing the exome or other areas of interest.

PS14.101

Sample processing for the UK Biobank Genotyping Project

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UK Biobank is a health research charity which has collected biological samples and a wide range of data on over 500,000 participants aged 40-69 years old. Data and samples are available for use by bona-fide researchers to investigate aetiology of disease. A genotyping project was initiated in 2013 to perform SNP genotyping on all UK Biobank participants within 18 months. The aim of the project - now 75% complete - is to generate high quality genotype data which is available for researchers to use, via UK Biobank.
DNA extraction and quantification is performed at UK Biobank; genotyping (on the custom UK Biobank Axiom® Array) is undertaken by Affymetrix; and the Wellcome Trust Centre for Human Genetics is performing QC, phasing and imputation on the derived data.
UK Biobank used the Trinean DropSense96 instrument for DNA quantification whilst Affymetrix used the PicoGreen method. This poster describes the sample processing workflow employed on the project and presents a comparison between the two quantification methods used. Genotyping metrics for a subset of the samples are also included.

PM14.102

Deciphering the atypical and discovering the unexpected in exome-sequencing using VarElect for phenotype-based variant prioritization

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Exome-sequencing is an effective tool to identify genes underlying Mendelian disorders. However, its routine clinical application is only beginning to emerge. A typical analysis generates a variant short-list often containing hundreds of candidates. To overcome the hurdle of connecting one variant to the patient's phenotype, we constructed VarElect, a new Variant Election software tool that attains phenotype-dependent variant prioritization, leveraging the comprehensive information within GeneCards and MalaCards. Users submit phenotype/disease related keywords and a gene list. VarElect then produces a prioritized list of contextually annotated genes, scored according to their likelihood to be disease related, thus enabling to perform the last decision step in NGS analysis in a fast and objective manner. In this realm, we diagnosed trichohepatoenteric syndrome in a girl harboring a novel mutation in *TTC37* (Oz-Levi et al. 2014).
In a second case, trio exome-sequencing of a girl with severe epileptic encephalopathy and her healthy parents revealed a novel missense mutation in *PCDH19* on chrX, causing epilepsy and mental retardation, limited to females (EFMR).
This disorder is showing a rare X-linked inheritance wherein an affected girl inherits a deleterious mutation through her unaffected father, a scenario that would not have been discovered by standard trio analysis that disregards such mutations on chrX. The *PCDH19* mutation was also found in the asymptomatic grandmother suggesting somatic mosaicism of neuronal cells

as a result of skewed X-inactivation leading to phenotypic variability. Our findings emphasis the impact of combining NGS with proper bioinformatics tools on discovery and diagnostics of the atypical and unexpected.

PS14.103

Development of a universal variant classification system and reporting scheme for routine NGS panel diagnostics for diagnostic purposes

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NGS is now widely used in routine molecular-genetic diagnostics and many laboratories have access to high-quality NGS data resulting in reliable variant detection. Guidelines [1] are currently formulated to standardize and harmonize many aspects of NGS testing with an emphasis on technical issues. Nevertheless, considerable inter- and intra-laboratory discrepancies concerning the classification and reporting of „variants of uncertain clinical significance" (VUS) can be observed.
There are several variant classification systems published. Some are restricted to specific genes or phenotypes (e.g. IARC [2]; HNPCC genes only), some are restricted to specific modes of inheritance (e.g. Ambry [3]; autosomal dominant and X-linked only), and most of them require extensive additional information (IARC, Ambry and Emory [4]; e.g. segregation data, functional assays, RNA data, immunohistochemical data, etc.) often unavailable at the time the report is created. We aimed to formulate a universal classification algorithm, allowing a robust and simple variant classification suitable for routine diagnostic laboratories.
Furthermore we created an algorithm for classification of variants which possibly affect RNA splicing, based on published data [5], [6], since these type of variants_although quite common_are not covered by the abovementioned systems.
Population frequency data from several exome sequencing studies and additional data (e.g. segregation analysis in families, clinical data, co-occurrence of pathogenic mutations, etc.) suggest that certain variants previously classified by the abovementioned classification schemes as pathogenic (e.g. truncating variants, variants affecting conserved splice sites, start-lost variants) should actually be classified as VUS [7]. To address this problem, we suggest a more stringent approach, classifying variants observed de novo as class 4 or 5.

PM14.104

The ABC of improving patient care and management in pediatric neurology by implementing next generation sequencing in routine diagnostic care

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Implementation of novel genetic tests into routine diagnostic practice is generally driven by technological advances as they promise to be faster and/or lead to increased diagnostic yield. Yet, other aspects including costs-to-diagnosis and the patients' information and communication needs are often not assessed prior to implementation. Here, we have addressed these aspects for the implementation of whole exome sequencing (WES) in pediatric neurology. We collected two cohorts (50 retrospectively and 100 prospectively selected patients) presenting with complex neurological problems of suspected genetic origin. Typically, finding a diagnosis in these patients is not easy, nor fast or cheap, and often involves burdensome procedures. In a unique parallel study design, all patients underwent both the conventional diagnostic procedure as well as WES. This unique set-up allowed for direct comparison of diagnostic outcomes, turn-around-times and costs involved. Analyses of the retrospective cohort indicated that WES identified significantly more conclusive diagnoses (10 *vs.* 3, p=0.04), using less genetic tests (1 *vs.* 7.72, p<0.01), in a shorter time (12 *vs* 40 months), and cost reduction for genetic testing (€4,372 *vs.* €5,321). In-depth interviews with parents pointed towards the need for more information and communication of the test and its results, and for more support in their daily struggles and concerns associated with living with a child with a rare genetic condition. Preliminary analysis of our prospective cohort confirms WES being superior to sequential genetic testing with regards to diagnostic yield. Our results provide essential information for an evidence-based implementation guideline of WES in pediatric neurology.
Financial support: ZonMW grant 40-41200-98-9131, The Netherlands.

PS14.105

TruePrime™, a novel technology for whole genome amplification from single cells and limited material

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TruePrime™ is a novel technology dedicated to the amplification of whole genomes and DNA from various sources. TruePrime™ is based on the combination of the highly processive Phi29 DNA polymerase with the recently discovered primase/polymerase TthPrimPol. In this setup, TthPrimPol synthesizes the DNA primers needed for Phi29 DNA pol, which allows for the exponential amplification of target DNA. TthPrimPol is a thermostable member of a recently discovered family of enzymes named PrimPol. TthPrimPol is a monomeric enzyme (34 kDa) that displays a potent primase activity, preferring dNTPs as substrates unlike conventional primases. This DNA primase activity can be activated by magnesium or manganese ions, having a wide sequence specificity for template recognition.
Key advantages of the TruePrime™ technology include complete absence of primer artefacts, insensitivity to external DNA contaminations, reduced amplification bias compared to methods using random synthetic primers, and an exquisite reproducibility when amplifying from single cells or minute DNA amounts. Moreover, TruePrime™ shows superior sensitivity, is easy to use and works perfectly well with commonly used NGS platforms such as Illumina or IonTorrent.
We believe that TruePrime™ will advance human genetic analyses from single cells or otherwise limited input material.

PM14.106

Standardising the approach to recruitment and phenotyping in rare diseases - experiences from the Genomics England Rare diseases programme

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We report on our experiences of developing eligibility criteria and phenotypic data models for recruitment of participants into the 100,000 genomes project in England.
The Genomics England Rare Diseases Programme will recruit and whole genome sequence (WGS) 50,000 English National Health Service (NHS England) patients with the aim of transforming diagnostic services and improving the understanding of rare human diseases.
While this approach is expected to improve the accuracy and speed of genetic diagnosis for patients, the reliable identification and interpretation of disease-associated genetic variants is challenging. The success of the project depends on the selection of participants with a high probability of having a disease with an underlying monogenic cause and the collection of detailed phenotypic data.
Therefore, the implementation of the Programme has required the development of clear guidelines regarding patient recruitment and the collection of phenotypic information. Given the complexity of rare diseases (>120 are currently included within the Programme) it has been necessary to provide guidance in a disease-specific manner. This has been achieved through the creation of disease-specific eligibility statements and phenotypic capture models, and a process for their curation. In particular, we will describe the value of the process as a means of defining best practice in the assessment and diagnosis of rare diseases and clarifying relationships between independent diseases, as evidenced by the frequent re-use of models.
We are confident that the creation of these resources will be one of the legacies of the Genomics England programme.

PS14.107

Implementation of a diagnostic clinical whole exome sequencing service with virtual panel analysis: experience at Guy's Genetic Centre, London

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Whole exome sequencing (WES) is becoming a frontline molecular diagnostic test in many Genetic Centres particularly for patients with heterogeneous disorders. To assess the feasibility of delivering an end-to-end Clinical WES service we have performed a pilot study of 96 patients over 6 months and describe our experience of the first 50 patients. Analysis using virtual panels restricts investigations to only clinically relevant genes, limiting the

chance of incidental findings whilst enabling panel flexibility for newly discovered genes.
Initially, 7 virtual panels were designed jointly by scientists and clinical geneticists with expertise in these disorders (dysmorphology, endocrine, neurology, ophthalmology, renal, skeletal & connective tissue disorders). Patients were phenotyped by clinical geneticists prior to testing using the Agilent SureSelect Human All Exon v5 kit on a HiSeq 2500. Variants detected within each virtual panel were filtered and those classified as potentially pathogenic were further assessed at a Multidisciplinary Team Meeting prior to verification by Sanger sequencing.
Clear pathogenic variants were identified in 26/50 (51%) of patients confirming their clinical diagnosis. The pathogenic variants identified spanned across a total of 27 different genes, include SNV, insertion/deletion and in 2 cases homozygous/hemizygous partial gene deletions. 12/27 (44%) of genes currently have no testing available on UK GTN including the PODXL gene, which was identified as a candidate gene for focal and segmental glomerulosclerosis (FSGS) in 2014.
These results show that a diagnostic WES service can achieve a high diagnostic yield in a clinically relevant time frame. We now plan to test another 150 patients.

PM14.108
Rapid screening for monogenic diseases in severely ill newborns and infants using whole genome sequencing

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Monogenic diseases are frequent causes of neonatal and infant morbidity and mortality. Routine molecular testing is time consuming and available for only few of these diseases. For severely ill newborns and infants quick molecular diagnosis is important for clinical decision-making and can prevent unnecessary and sometimes invasive diagnostics.
Here we describe a procedure and present the first results to analyze 2800 genes in severely ill newborns and infants by rapid whole-genome sequencing (WGS). The procedure is carried out by a multidisciplinary team of pediatricians, clinical geneticists, technicians, laboratory specialists, researchers and bioinformaticians. Thus far we have included 11 patients and analyzed them with a turnaround time of approximately one week. We have provided a diagnosis of a monogenic disease for one patient who presented with microcephaly, seizures and developmental delay and appeared to have compound heterozygous mutations in the *EPG5* gene which is associated with Vici syndrome.We retrospectively tested 4 of the patients without diagnosis using rapid clinical exome sequencing in trio design, focusing on the same set of 2800 genes, resulting in no additional diagnoses. We additionally tested 5 patients who died within the first year of life using clinical exome sequencing in trio design to further test this procedure. We found compound heterozygous mutations in *BRAT1* in one child with an unexplained severe seizure and rigidity disorder.

PS14.109
Whole genome sequencing of human saliva samples within the Deciphering Developmental Disorders (DDD) study

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The DDD exome pipeline routinely generates high quality DNA sequence data from saliva samples. Here we investigate the suitability of saliva DNA for whole genome sequencing (WGS). Our aim was to evaluate the potential for off-target mapping caused by the presence of bacterial DNA in the sample.
We whole genome sequenced 30 DDD trios (proband, mother, father) to an average depth of 30X on the Illumina X10 HiSeq platform. Parental DNA was extracted from saliva collected in Oragene tubes (DNA Genotek), while proband DNA was extracted from either blood or saliva.
Bacterial qPCR was performed as described by DNA Genotek (PD-PR-065). The assay utilises "universal" bacterial primers targeting a well-conserved region of the 16S ribosomal RNA gene. The median percentage of bacteria was 5.5% in adult saliva, 27.7% in child saliva and 0.02% in blood. We compared the percentage bacteria per sample to the percentage of reads mapping on-target by WGS. We discovered a high level of correlation between these two parameters. The minimum depth per sample was 10X (35% mapping), and the median depth 27X (90% mapping). Therefore even in samples with a high percentage of bacteria, a sufficient proportion of reads are mapped such that variant detection should be possible.
We find bacterial qPCR to be a robust and scalable method for screening samples for the presence of bacterial DNA. While it may not encompass the full spectrum of non-human DNA (e.g. viral, fungal), it provides a cost-effective method for detecting and filtering out poor candidates for WGS.
Grant: Health Innovation Challenge Fund (HICF-1009-003)

PM14.110
Towards gene correction of IVS1-110 β-thalassaemia

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Thalassemia is amongst the commonest single-gene disorders worldwide and caused by deficient production of α- or β-globin. The disease has limited curative treatment options, but as a monogenic disorder of the hematopoietic system is ostensibly an ideal target for gene therapy. Specific β-thalassemia mutations have already been addressed by the burgeoning field of genome editing, targeting mutations of minor prevalence in the West and combining homology-mediated gene repair with the expansion of corrected iPS cell clones
Our research is focused on the development of efficient gene-correction tools (TALENs and CRISPR-Cas9) specific for the common and severe HBBIVS1-110 (G>A) mutation, which in most Mediterranean and many Western countries has a frequency of above 20% (with 80% on the island of Cyprus) amongst β-thalassemia carriers. This mutation introduces an abnormal splice acceptor site in intron 1 of the β-globin gene, therefore retaining an intronic in-frame premature stop codon in the mature, aberrantly spliced mRNA.
In this study, we illustrate the high cleavage activity of novel IVS1-110-specific genome-editing tools (TALENs and CRISPR-Cas9) in HEK293T cells and in HBBIVS1-110-transgenic murine erythroleukaemia cells. Towards the assessment of therapeutic efficiency of our designer nucleases at the mRNA level, we have validated a multiplex RT-qPCR method for the absolute quantification of the correctly and aberrantly spliced HBB mRNAs. Finally, immunoblots indicate the partial correction of β-globin protein expression in treated HBBIVS1-110-transgenic MEL cells compared to mock controls. These preliminary data indicate HBBIVS1-110 as a suitable target for gene therapy genome editing and that our approach may serve as a model for the correction of many other intronic disease-causing mutations.

PS15.01
A patient with 4q13-q22 chromosomal deletion and Incontentia Pigmenti skin lesion. Diagnostic problems and therapeutic options

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Background: Deletions of the 4q13-q22 chromosomal region have infrequently been reported. The patients may be short, microcephalic and severely retarded with multiple minor anomalies. Two of the three patients reported died before 11 months of age.
Incontinentia pigmenti (IP) is a genodermatosis that segregates as an X-linked dominant disorder and is usually lethal prenatally in males. In affected females it causes highly variable abnormalities of the skin, hair, nails, teeth, eyes, and central nervous system.
Exome sequencing is a technique for sequencing all the protein-coding genes in a genome.
Material and Methods: A new born girl with some dysmorphic features developed a skin rash, cephalic hematoma and seizures. She was treated with antiepileptic drugs.
MRI and EEG were done as well as chromosomal analysis, Array comparative genomic hybridization (ACGH) and exome sequencing.
Results: Chromosomal analysis revealed the 4q13.1-22 deletion while ACGH defined the breakpoints. The skin lesion was compatible with IP.
Exome sequencing was done to look for variants in the remaining allele of the deleted genes at 4q looking for variants of importance for her phenotype and thus hopefully give possible therapeutic options. The exome results are pending.
Conclusion; 4q13-q22 deletion and Incontinentia pigmenti (IP) have never been described in the same patient and it is the first large 4q13-q22 deletion case with identified breakpoints.

To our knowledge this is the first case with a defined chromosomal deletion where exome sequencing was done to investigate the remaining allele of the deleted genes looking for possible therapeutic options.

PM15.02
Swedegene: genome-wide association study of drug-induced agranulocytosis

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Introduction: Agranulocytosis is a rare, serious condition with a case-fatality rate of 7-10%. It can be caused by a multitude of drugs, and genetic factors are believed to play a role in the pathogenesis. Swedegene (www.swedegene.se) is collecting patients with drug-induced agranulocytosis on a nation-wide basis. We aim to discover genetic and clinical factors that predispose to drug-induced agranulocytosis to enable personalised treatment in the future.
Materials and Methods: A genome-wide association study was performed on 94 adjudicated Swedish agranulocytosis cases and 4891 unrelated controls from TwinGene. Cases were genotyped with Illumina HumanOmni2.5-8 and controls with Illumina HumanOmniExpress-12v1 at Uppsala University SciLife SEQ&SNP Technology Platform. Analyses were performed using PLINK and HLA imputations using SNP2HLA. After quality control, the merged genotype set contained ~600k SNPs. The significance level was set at p<8.39x10-08.
Results: The main causative drugs were sulfasalazine, antithyroid agents and antibiotics. Statistical analyses identified significant associations in the HLA region on chromosome 6. After HLA genotype imputation, the strongest signal was HLA-C*02:02 (OR [95% CI] = 3.37 [2.25, 4.92], p=8.75x10-10). The HLA locus remained significant when utilising 233 controls matched for disease and/or treatment.
Conclusions: We found an association between drug-induced agranulocytosis and the HLA locus. We are proceeding with additional cases and controls obtained through collaboration with Spain, France and Germany to strengthen the finding. Collaborators with replication cohorts are invited.
Grants: Swedish Research Council, Heart-Lung Foundation, Society of Medicine and Medical Products Agency, Uppsala University Clinical Research Support (ALF) and Uppsala County Council Research Fund.

PS15.03
CYP2D6 enzymatic deficiency and weight gain in patient treated with atypical antipsychotics

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Introduction. Several atypical antipsychotic drugs are associated with metabolic side effects including weight gain, body mass index (BMI)/blood Insulin level increase. The half-life of atypical antipsychotics varies depending on the activity of the CYP2D6 enzyme, but there is lack of data regarding the impact of CYP2D6 isoenzyme polymorphisms on the long-term effects, in paediatric patients using atypical antipsychotics.
Aim. In this study we aimed to underline the role of individual genetic variation, in correlation with the CYP2D6 genotype, for weight gain in patients treated with atypical antipsychotics.
Material and methods. The study lot included 80 patients, aged between 9 and 20 years, median age being 15.75. The sex percentage in the sample was 55% girls / 45% boys. All the patients were receiving one of the choosen atypical antipsychotic-Risperidone, Aripiprazole or Olanzapine. We evaluated the BMI and the blood insulin variations for these patients in different time points during the treatment with atypical antipsychotics. CYP2D6*3, *4, *5, *41 allele identification was performed.
Results. Based on the CYP2D6 genotype, three activity groups were identified and compared. The CYP2D6 genotype in children and adolescents with schizophrenia and bipolar disorder, proved to be a good predictor for the risk of gaining weight, metabolic ratio and the increase of insulin blood level.
Conclusion. The significant correlations between the CYP2D6 polymorphisms and the weight gain/BMI and/or blood Insulin increase, as major side effects induced by antipsychotics proved the fact that the pharmacogenetic screening is needed in the future clinical practice, allowing for individualized treatment.

PM15.04
Using genetic and gene expression methods to identify biological predictors of response to cognitive behavioural therapy for anxiety disorders

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Anxiety disorders are the most prevalent group of psychiatric disorders, and represent a major global burden both to the individual and to the state. Cognitive behavioural therapy (CBT) is commonly used to treat anxiety, and is effective in children and adults. However, a substantial proportion of those receiving CBT fail to remit following treatment. The expense and distress of ineffective treatment is good justification for seeking predictors of response.
Genome-wide methodologies present a hypothesis-neutral strategy for exploring the potential of genetic variants as predictors. We have performed GWAS in a global study of predictors of response to CBT in children with anxiety disorders (the Genes for Treatment study, N=980). Although no variants reach conventional levels of genome-wide significance for response immediately post-treatment, or at a six-month follow-up, seven loci are suggestive of significance.
The results of the GWAS suggest that individual genetic variants are unlikely to predict useful amounts of variance in response. However, polygenic approaches may be valuable. We have used an independent cohort of adults receiving CBT for anxiety disorders (N=200), to perform polygenic risk scoring to predict response.
Genetic variants do not exist in isolation. Using our adult cohort, we have undertaken a combined analysis of genome-wide genetic and gene expression data. In addition to identifying cis-eQTLs for differentially-expressed transcripts, we have created modules of genes using literature-driven and data-driven modalities, and derived principal components from these to test whether genetic variants can be identified that drive the expression of relevant pathways.

PS15.05
Non-invasive genomic profiling of bladder cancer using urinary cfDNA

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Urothelial bladder cancer (UBC) is the 7th most common cancer in Western societies with a rising global incidence. UBCs are highly heterogeneous in their clinical characteristics, and this is mirrored in the often highly complex genomic profiles observed in formalin-fixed paraffin-embedded (FFPE) tumour material. Identification of such complex genomics and detection of important specific genomic biomarkers in a non-invasive fashion would be highly advantageous for diagnosis, treatment selection and monitoring of disease.
Here we show genomic data from >100 FFPE UBC tumour samples profiled using the OncoScan® FFPE assay kit. The samples were obtained from a well characterised UBC patient cohort with >3 years clinical follow-up. Analysis of this data reveals novel predictive and prognostic genomic biomarkers in UBC. Additionally we show data from concurrent, parallel genomic analyses of 23 UBC patients using DNA extracted from urine cell pellets; cell-free DNA (cfDNA) from urine supernatants, and DNA from FFPE tumour samples. This data illustrates the potential of DNA extracted from the urine for non-invasive genomic profiling of UBC and for the identification of predictive and prognostic biomarkers. Interestingly our data shows increased sensitivity of cfDNA (85%) over DNA from urinary cell pellets (55%) for accurate non-invasive genomic profiling of UBC.

PM15.06
Alteration in the PI3K/AKT signaling pathway in Czech breast cancer patients

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Introduction: Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death in females worldwide. Deregulation of PI3K/AKT signaling pathway has been associated with cancer development and progression and is promising target for breast cancer therapy.
Materials and Methods: DNA samples of breast tumour tissues were tested for presence of selected somatic mutations in PIK3CA and AKT1 genes. Analysis was performed by primer extension method or real-time PCR. Statuses of HER2 gene and ER/PR expression were evaluated by FISH and/or immunohistochemistry methods using FFPE tissue sections. Correlation between PIK3CA mutation status and clinicopathological characteristics were estimated with the chi-squared test (95% CI).
Results: Somatic mutations in PIK3CA gene were detected in 26,1% (63/241). Mutations in the exons 9 and 20 were detected in 36,5% and 57,1%, respectively. Coexistence of mutations (exon 9 and 20) was found in 6,4%. Mutation p.E17K in AKT1 gene was detected in 1,7% (2/115). PIK3CA mutations were signicantly associated with low histopathological grade and ER positive status.
Conclusions: Frequency of PIK3CA and AKT1 gene mutations correlates with published data. Marked differences in distribution of PIK3CA mutations were found among breast cancer subtypes (ER+ , HER2- and triple negative). Alterations involving the PI3K/AKT pathway may have distinct prognostic and predictive impacts on patients. Novel combination of treatment strategies, involving PI3K pathway inhibitors, has a potential to improve clinical benefit of breast cancer patients.
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PS15.07

Association of Twist transcription factor expression and chemosensitivity of primary breast tumor samples

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Doxorubicin and Docetaxel are anticancer agents which commonly used as a part of breast cancer chemotherapy regimens. TWIST1 (OMIM 601622) gene encodes Twist transcription factor which is a basic-helix-loop-helix protein and regulates the mesoderm formation and differantiation during development. In vitro studies using cancer cell lines sugessted that Twist transcription factor might have a role in resistance to chemotherapeutic agents in some cancer types including breast cancer. We aimed to investigate the association of twist gene expression levels and theraphy response to doxorubicin and docetaxel in primary breast tumor samples. 26 tumor samples were collected from the patients. Chemosensitivity of primary breast tumor samples to doxorubicin and docetaxel have been determined by ATP based tumor chemosensitivity assay. Total RNA was extracted by using RNeasy Mini Kit (Qiagen) from tumor samples. Twist gene expression of tumor samples were determined by Real Time Reverse Transcriptase PCR by using beta actin gene expression as reference. (Real Time Ready Gen Expression Assay, Roche) 9 out of 26 tumors were nonresponsive to doxorubicine whereas only 3 out of 26 tumors were nonrespnsive to docetaxel. Doxorubicine non-responsive breast tumors had higher TWIST1 gene expression levels compared to doxorubicine responsive breast tumors (p=0.041) There were no association of twist gene expression with docetaxel resistance (p=0.3). In conclusion our results support that twist transcription factor might be a candidate biomarker to predict chemoresponse of breast tumors to doxorbicine. Further studies with larger sampe groups are needed to clarify the role of Twist gene expression in resistance to chemotheraphy.

PM15.08

Clinical validation of a NGS based in vitro diagnostic (CE-IVD) kit for targeted detection of actionable gene rearrangements in lung cancer specimens

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In recent years, advances in next-generation sequencing (NGS) technologies have enabled faster and cheaper methods for uncovering the genetic basis of disease. For cancer, NGS based screening for known tumor subtypes can inform diagnosis and allow the clinician to tailor a specific therapy based on testing outcome. Here we present the validation of one such NGS based kit approved for CE-IVD use to screen for specific chromosomal translocations in non-small cell lung cancer (NSCLC) samples by targeting specific breakpoints in known fusion transcripts.
The kit tested (Oncomine™ Solid Tumor Fusion Transcript Kit) included a single primer pool containing amplicon designs to simultaneously screen for over 75 specific rearrangements involving the receptor tyrosine kinase (RTK) genes ALK, RET and ROS1 as well as NTRK1. The panel was compatible with formalin-fixed paraffin-embedded lung tumor samples and achieved high-sensitivity down to 10 ng of RNA input. In addition, amplicon assays designed at the 5’ and 3’ ends the RTK genes provide non-specific evidence that a translocation exists in a sample by comparing expression imbalance between the two ends.
Validation testing was carried out at three external clinical laboratories (CLIA, CAP, INAB). In addition to positive and negative control samples, each site contributed FFPE lung tumor samples for which ALK fusion status was known prior to NGS library preparation carried out using the Ion AmpliSeq™ workflow. For site-specific samples samples (n=144), high concordance, sensitivity and specificity were measured at 97.2%, 90.5% and 98.4% respectively.

PS15.09

Effect of CYP2D6 genotypes on the pharmacodynamics of carvedilol in healthy korean volunteers

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Depending on the variation of genes related with the drug metabolism, a drug may express no effect or cause severe adverse effect. For this reason, MFDS, Korean national drug regulatory authority put great effort to harness pharmacogenomics using genomic information from Korean population.
As a part of this effort, the pharmacodynamic study of carvedilol, a important beta blocker widely used in Korea, has been conducted to find out the effect of CYP2D6 genotype on pharmacodynamic endpoints of carvedilol. After recruiting CYP2D6 genotype-specific subjects(EM, IM1 and IM2), a clinical study was conducted to check pharmacodynamic endpoints.
Heart rate, systolic blood pressure and diastolic blood pressure were decreased by medication of carvedilol in all genotype groups. Isoproterenol sensitivity test (IST) also showed increased CD25 after the medication in all genotype groups like other vital signs.
The mean CD25 value showed no statistically significant change when compared with basal level. But the mean CD25 value between different genotype groups (EM vs IM) revealed some statistic singificance ($p < 0.05$).
The pharmacodynamic study of carvedilol in Korean populations showed that CYP2D6 genotype caused difference in CD25 value of IST but not in major clinical endopoint such as heart rate, systolic blood pressure and diastolic blood pressure. These results inferred that carvedilol does not cause any important clinical difference depending on the CYP2D6 genotype. In conclusion, these results suggested that dose adjustment does not requried for the carvedilol treatment in Korean population.
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PM15.10

Development and analytical validation of an ADME gene Ion AmpliSeq sequencing assay covering 143 SNP and CNV Pharmacogenetics targets.

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Cytochrome P450 (CYP) enzymes metabolize about 75% of drugs, with UGT enzymes metabolizing about another 15%. Variations in gene sequence or copy number may result in an inactive, defective, unstable, mis-spliced, low expressed, or absent enzyme, an increase in enzyme activity, or an altered affinity for substrates. ADME gene genotypes can predict whether an indi-

vidual is a poor or rapid metabolizer, facilitating dose optimization to avoid adverse or insufficient drug reaction.
We have designed a pharmacogenomics (PGx) Ion Ampliseq panel to detect 143 variants in 37 ADME genes including copy number detection in CYP2D6, covering the common, actionable, interpretable and reimbursable targets in genes encoding drug metabolism enzymes and associated transport proteins, including CYP2D6 and CYP3A4. The panel contains 134 amplicons in an ultrahigh-multiplex PCR in a single pool, followed by Ion Torrent™ semiconductor sequencing; the assay is compatible with FFPE samples as well as non-FFPE samples, requiring as little as 10 ng of input DNA. This panel can be customized, allowing additional targets to be added.
Analytic validity of the panel was established by sequencing 90 annotated cell lines from Coriell, and comparing the Ion AmpliSeq genotypes to gold standard TaqMan OpenArray genotypes. Concordance with TaqMan genotypes was > 99.8%, genotype reproducibility was > 99.9%, and the genotype no-call rate was < 0.15%.
These results demonstrate an ADME genotype assay with high accuracy, which can be used to explore potential pharmacogenomic relationships, including the relationship between copy number and genotype of DME genes on drug tolerability and clinical outcomes.

PS15.11
High frequency and founder effect of the CYP3A4*20 loss-of-function allele in the Spanish population classifies CYP3A4 as a polymorphic enzyme

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Cytochrome P450 3A4 (CYP3A4) is a key drug metabolizing enzyme. Loss-of-function variants have been reported as rare events, and the only demonstration of a CYP3A4 protein lacking functional activity is caused by *CYP3A4*20* allele. Here we characterized the world distribution and origin of *CYP3A4*20* mutation. *CYP3A4*20* was determined in more than 4000 individuals representing different populations and haplotype analysis was performed using *CYP3A* polymorphisms and microsatellite markers. *CYP3A4*20* allele was present in 1.2% of the Spanish population (up to 3.8% in specific regions), and all *CYP3A4*20* carriers had a common haplotype. This is compatible with a Spanish founder effect and classifies CYP3A4 as a polymorphic enzyme. This constitutes the first description of a *CYP3A4* loss-of-function variant with high frequency in a population. *CYP3A4*20* results together with the key role of CYP3A4 in drug metabolism, support screening for rare *CYP3A4* functional alleles among subjects with adverse drug events in certain populations. Currently, we are genotyping *CYP3A4*20* allele in Spanish individuals treated with various CYP3A4-substrates to better characterize the pharmacokinetics of *CYP3A4*20* carriers.

PM15.12
Role of pharmacogenetic on deferasirox AUC and efficacy

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Deferasirox is a once-daily oral administered iron chelator and its pharmacokinetic significantly correlates with therapy outcome. This drug is metabolized in liver by UDP-glucuronyltransferase (UGT) 1A1 and 1A3, by cytochrome-P450 (CYP) 1A1, 1A2 and 2D6 enzymes, and it is eliminated via biliary-enteric circulation through multidrug resistance protein 2 (MRP2).
We perform a retrospective study of deferasirox pharmacokinetic (AUC, area under the curve) according to single nucleotide polymorphisms (SNPs) in genes involved in this drug metabolism and elimination, in a cohort of adult β-thalassemic patients. Moreover, we define a plasma AUC cut-off value predicting an adequate response to therapy.
Allelic discrimination for SNPs in UGT1A1, UGT1A3, CYP1A1, CYP1A2, CYP2D6, MRP2 and BCRP1 genes is performed by real-time PCR. Drug plasma concentrations are measured by an HPLC-UV validated method. AUC over 24 hours values are determined by the mixed log-linear rule, using Kinetica software.
Sixty patients meet the inclusion criteria. UGT1A1 CT/TT (rs887829), UGT1A3 TT (rs1983023) and AG/GG (rs3806596) SNPs show an influence on the half-life (p=0.029, p=0.043 and p=0.043 respectively); UGT1A3 GG (rs3806596) and CT/TT (rs1983023) SNPs significantly influenced AUC, volume of distribution, maximum serum concentration and time of maximum concentration. According to Chirnomas effectiveness definition, a deferasirox AUC cut-off value of 360 ng/mL/h was identified (ROC-curve, p=0.012). UGT1A1 rs887829 (p=0.008) and ABCG2 rs13120400 (p=0.007) SNPs are factors able to predict concentration above 360 ng/mL/h in the logistic regression analysis. These data contribute to a better management of deferasirox treated patients, suggesting the usefulness of a genetic-based dose personalization.

PS15.13
Harnessing publicly available genetic data to prioritize therapeutic targets based on adverse risk profile

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LDL-cholesterol (LDL-C) reduction effectively reduces risk of coronary artery disease (CAD). However, statins (the most widely prescribed LDL-C lowering drugs) increase type 2 diabetes (T2D) risk. Using public domain data from genome-wide association studies, we conducted genetic epidemiological investigations to identify druggable loci that alter LDL-C and CAD risk without causing dysglycemia. A one standard deviation (SD) increase in LDLC caused an increased odds ratio (OR) for CAD of 1.63 (95% confidence interval [CI]: 1.55, 1.71) and LDL-C/CAD-associated SNPs showed consistent effect directions (binomial P=4.93x10-21). A 1-SD higher LDL-cholesterol was protective of T2D (OR 0.86; 95%CI:
0.81, 0.91), however LDL-C/T2D- ssociated SNPs didn't show consistent effect directions (binomial P=0.08). PCSK9, APOB, LPA, CETP, PLG and ALDH2 were identified as druggable loci that alter LDL-cholesterol and CAD risk without causing dysglycemia, indicating drugs targeting these gene products may reduce CAD risk without increasing T2D risk.

PS15.15
Genomic risk prediction of obesity and related disorders: body mass index vs. waist-to-hip ratio

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Introduction: Waist-to-hip ratio (WHR) has been suggested to be a better predictor of obesity and related disorders than body mass index (BMI). However, it is unclear which of the two are most appropriate for genomic risk stratification. The aim of the study was to test whether predicted genomic values (PGV) for WHR are better than those of BMI in classifying outcomes for obesity and related disorders within a Croatian (N=2,159) and into a UK (N=805) population sample. Materials and Methods: PGV were estimated in the genomic best linear unbiased prediction (GBLUP) and Bayes C framework. The discriminative power of BMI and WHR PGV in classifying outcomes for general (BMI ≥30 kg/m2) and abdominal (WHR >1.0 in men and >0.85 in women) obesity and related disorders (chronic obstructive pulmonary disease, hypertension, peripheral vascular disease and metabolic syndrome) was assessed by the area under the receiver operating characteristic curves (AUC) and bootstrap-derived confidence limits. Results: Performance of GBLUP prediction was similar to that of Bayes C in both populations; suggesting that the genetic architecture of BMI and WHR approximates the infinitesimal model. BMI classified genomic risk of obesity and related disorders as well as or better than WHR. All AUC reported in this study ranged from 0.51 to 0.81; indicating low to moderate discriminatory value. Conclusions: Inclusion of PGV in combination with the traditional risk

factors (age, age2 and sex) in most cases augmented the AUC; indicating that genomic information can be used to supplement traditional risk factors in prediction models.

PM15.16

BRAF mutations in Slovak cohort of patients with gastrointestinal stromal tumors

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Introduction: Gastrointestinal stromal tumors (GISTs) are highly resistant to conventional chemotherapy and radiotherapy, however sensitive against tyrosine kinase (TK) inhibitors. Approximately 10 to 15% of GISTs are lakking the mutations in KIT and PDGFRA and are referred as wildtype GISTs (wtGISTs) which are less sensitive to TK inhibitors. One of the reasons can be the activated RAS-BRAF signaling.
Material and methods: Formalin-fixed paraffin-embedded (FFPE) tissue sections were collected as a part of the National GIST Registry from 705 patients with the diagnosis of GIST in years 2004-2014 and were genetically characterized for KIT and PDGFRA mutations by dideoxysequencing - 150 patients' samples corresponded to wtGISTs and further 100 patients with mutation in KIT or PDGFRA were included in the study. For the detection of BRAF V600E, dideoxysequencing and allele-specific PCR were used.
Results: The proportion of the BRAF-positive patients (V600E and K601N) in the total cohort of patients with GIST analyzed for the mutational status in KIT and PDGFRA genes is 1.42% (10/705), in the wtGIST subset 6.67% (10/150). Only in 5 cases, the BRAF mutation could be confirmed by dideoxysequencing showing the low amount of BRAF positive cells in the tumor mass. Two patients of hundred previously identified as mutated in KIT or PDGFRA have harbored the BRAF mutation too.
Conclusion: BRAF mutations may affect the response to imatinib of wtGIST and KIT imatinib-sensitive mutations representing new mechanisms of primary resistance to targeted therapy in GIST. The BRAF testing in GIST should be taken into account.
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PS15.17

Association of polymorphisms in DNA repair genes with disease-free survival in patients diagnosed with head and neck cancer

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Introduction: Head and neck cancer represents one of the most common cancers in the developed world. The main risk factors (tobacco, alcohol, etc.) can cause DNA damage as well as cancer treatments. Therefore, polymorphisms in DNA repair genes may alter the ability to repair DNA damage increasing the susceptibility to develop the disease and modifying the response to treatment and survival. In this work we focused on the influence of these polymorphisms on the disease free survival.
Methods: We studied 173 patients with head and neck cancer. *XRCC1* rs25487, *APEX1* rs1130409, *XPD* rs13181, *ERCC1* rs11615 and *XRCC3* rs861539 polymorphisms were genotyped. Statistical analysis was performed according to the different models of inheritance (codominant, dominant, recessive, additive). We stratified the analysis by stages at diagnosis.
Results: We found an increased risk of relapse in patients with minor alleles of *XPD* or *ERCC1* (Table 1) in 78 patients with localized disease (stages I and II). Regarding patients with advanced disease (stages III and IV), we studied a total of 95 patients and we did not find any statistically significant results.
Conclusions: We found an association of *XPD* (rs13181) and *ERCC1* (rs11615) variants with disease free survival depending on the stage at diagnosis. These results support the importance of the nucleotide excision repair pathway in these tumors.
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Table 1. Genotypic distribution in patients with local disease.

Genotype	No. patients	No. events	HR	95%CI	p-value
XPD codominant					
AA	30	8		Reference	
AC	36	16	2.07	0.88-4.86	0.094
CC	12	8	3.80	1.41-10.26	**0.008**
XPD dominant					
AA	30	8		Reference	
AC+CC	48	24	2.43	1.09-5.44	**0.031**
XPD recessive					
CC	12	8		Reference	
AA+AC	66	24	0.40	0.18-0.91	**0.028**
XPD additive					
AA/AC/CC	78	32	1.95	1.19-3.18	**0.007**
ERCC1 codominant					
TT	31	8		Reference	
TC	34	18	2.65	1.11-6.36	**0.029**
CC	13	6	2.36	0.79-7.02	0.124
ERCC1 dominant					
TT	31	8		Reference	
TC+CC	47	24	2.57	1.11-5.98	**0.028**
ERCC1 recessive					
CC	13	6		Reference	
TT+TC	65	26	0.77	0.31-1.88	0.566
ERCC1 additive					
TT/TC/CC	78	32	1.56	0.97-2.51	0.064

PM15.18

Resequencing of GWAS loci identifies new candidate genes on chromosome 5 for heparin-induced thrombocytopenia

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Treatment with unfractionated (UFH) or low molecular weight heparins (LMWH) represents a mainstay for the prevention and treatment of thrombotic disorders. Heparin-induced thrombocytopenia (HIT) is a potentially deleterious complication of heparin use. To explore whether a genetic predisposition contributes to HIT, we performed a pharmacovigilance study enrolling patients with suspected HIT and corresponding control patients. Subsequently, we selected 182 cases and 182 controls for the pharmacogenetic substudy consisting of a genome-wide association study (96 cases) with replication in additional 86 cases, followed by imputing and overall fine mapping analysis. One single nucleotide polymorphism (SNP, rs1433265) from initially 16 identified SNPs was successfully replicated (P=1.5x10-4) and remained the most strongly associated SNP (P=3.5x10-5) after imputing genotypes on chromosome 5. Fine mapping revealed two significantly associated haplotypes with an odds ratio of 0.63 (95% CI, 0.46-0.88; P=5.6x10-3) and 2.41 (95% CI, 1.64-3.55; P=4.9x10-6).In order to find variants not detected in our GWAS but contributing to the association signals, we applied a NGS-based targeted resequencing approach in a subgroup of 73 HIT patients and 23 controls for the regions with the 16 most strongly HIT-associated SNPs. A C-alpha test was applied to perform a gene-based test for the impact of rare variants in our targeted region and we were able to detect two associated HIT-candidate genes, ICE1 (P = 0.010) and ADAMTS16 (P= 0.005) containing 17 and 23 rare variants, respectively. These results provide a basis for further studies that aim to characterize the genetic predisposition to HIT.

PS15.19

IFNL4 polymorphism is a predictor of hepatitis C treatment efficiency in Ukrainian patients

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The discovery of host genotype impact in combination with virus genotype on chronic hepatitis C (CHC) treatment outcome was a milestone in the development of antiviral therapy strategies. The aim of this study was to examine association between IFNL4 (interferon-lambda 4) gene ss469415590 and treatment efficiency in group of Ukrainian PEG-interferon-treated CHC patients.
Study group was 92 unrelated HCV genotype 1 mono-infected CHC patients. Viral load was detected at weeks 4, 12, 24, 48 and 72. The patients were distributed into: case group - 29 patients with late or absent viralogical response, and control group - 63 patients with sustained viralogical response (SVR). Study material - genomic DNA extracted from peripheral blood. Genotyping for IFNL4 gene ss469415590 was performed using amplification-refractory mutation system PCR. Statistical analysis was performed using GenePop and OpenEpi statistical packages. A P-value of less than 0.05 was

regarded as significant.
Frequency of ss469415590 ΔG carriers was significantly higher in patients without SVR (86.2%) comparing to group with SVR (50.8%). Obtained results imply ss469415590 TT/TT genotype positive association with SVR, whereas ss469415590 ΔG/ΔG genotype is associated with poor virological response. This association fits into additive model of inheritance, ss469415590 ΔG/ΔG homozygotes have 3,6-times higher risk of poor response to PEG-interferon/ribavirin combination therapy (OR=3,62; CI95%: 1.12-11.67).
Our study presents the evidence of ss469415590 being informative pharmacogenetic marker of CHC treatment efficiency in Ukrainian patients.
This work was supported by the NAS of Ukraine [# 0112U002108]; and the President of Ukraine's grant for talented youth [# 72/43].

PM15.20
The plant cytokine kinetin as a potential therapeutic agent to correct CFTR splicing defects

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Cystic fibrosis (CF) is a common recessive disorder caused by >1900 mutations in the CF transmembrane conductance regulator (CFTR) gene. About 13% of CFTR mutations are classified as splicing defects. Besides these severe mutations, other variations are known to modulate the correct CFTR splicing, the most important being the polymorphic TG(9-13)T(5,7,9) locus, which influences exon-10 inclusion and has been associated with monosymptomatic forms of CF. Recent advances in CF treatment have demonstrated the efficacy of drugs targeting specific classes of molecular defects, such as potentiators (Ivacaftor, already FDA approved and suitable for about 4% of patients) and correctors (Vx-661 and Vx-809 for F508del), opening a new era of personalized treatment. However, no such therapies are available for patients carrying splicing mutations.
For this reason, we are exploring the possibility to improve exon-10 inclusion in CFTR mRNA using the plant cytokinin kinetin, an FDA-approved drug previously found to correct aberrant splicing in familial dysautonomia and neurofibromatosis.
We tested the effect of kinetin on CFTR splicing in Caco-2 cells, which express high level of CFTR transcript and present a significant amount of exon-10 skipping.
By competitive fluorescent RT-PCR and digital RT-PCR we demonstrated that different concentrations of kinetin determine a dose-response rescue of wild-type CFTR splicing. These results reveal a remarkable impact on splicing fidelity by this small molecule, which may represent a promising therapeutic agent for CF and particularly atypical forms. Moreover, it will be interesting to test whether kinetin treatment can also partially rescue severe splicing mutations in CFTR.

PS15.21
Do metabolic disease genes SNPs affect elite sports performance?

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Introduction: Complex metabolic diseases and physical activity levels are closely-related. PPAR family and members of the PGC-1 are key regulators of energy homeostasis and metabolism. We studied the association of PPARGC1A rs8192678, PPARGC1B rs7732671, PPARA rs4253778, PPARD rs2016520, PPARG rs1801282 which have been shown to impact metabolic dysfunction and elite athletic status. We hypothesised the metabolic risk genotype/allele to be underrepresented in elite athletes (n=130, sprint/power and endurance orientated) compared to controls (n=175, healthy unrelated non-athlete Lithuanians). Methods: Genotyping was performed by PCR-RFLP. Results: The genotypes' distribution was in HWE within all groups. For three SNPs the genotype frequencies were significantly different between the total athlete and control group (PPARGC1B CC/CT/TT: 89.2/10.8/0% vs 80.6/19.4/0%; P=0.04; PPARD TT/TC/CC: 88.5/11.5/0% vs 78.9/19.4/1.7%; P=0.05; PPARA GG/GC/CC: 61.5/38.5/0% vs 70.3/26.9/2.9%; P=0.02). PPARA genotype distribution in sprint/power athletes significantly differed from controls (GG51.4; GC48.6; CC0%; P=0.03). The PPARA C allele in the sprint/power athletes (24.3%) was more frequent compared to endurance (17.7%) and controls (16.35%). Having the metabolic risk-related PPARA GC&CC genotypes increases chances by 2.23 times (95%CI, 1.07-4.67) of achieving better results in sprint/power sport. The other SNPs did not show significant differences between the study groups. Conclusions: we found an association between PPARA rs4253778 and athletic status. Sprint/power athletes are more likely to have the metabolic risk allele C of PPARA compared to controls. These results suggest that some SNPs across the human genome have dual effect and may predispose sprint/power athletes to increased risk of developing metabolic morbidities compared with the general population.

PM15.22
NRAS genotyping in patients with metastatic colorectal cancer

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Introduction: Metastatic colorectal cancer (mCRC) is the second cause of dead in the world. Development of monoclonal antibodies against Epidermal Growth Factor Receptor (EGFR), Cetuximab and Panitumumab, has increased the survival of patients with mCRC. The efficiency of these drugs depends on the use of genetic biomarkers such as KRAS and, recently, NRAS. It has been found that patients with mutations in these genes have poorer survival rate when they are treated with anti-EGFR therapy. The objective of this study is to perform a mutational analysis of NRAS in patients with mCRC, and correlate the findings with the response to anti-EGFR drugs.
Material and Methods: We performed mutational analysis by pyrosequencing to detect the commonest changes in codons 12, 13, 59, 61, 117 and 146 of NRAS, in tumours samples of 493 patients with mCRC treated with anti-EGFR drugs, and whose KRAS mutational status has been studied previously. We also compared the percentage of mutations detected in the different codons of KRAS and NRAS with previous works.
Results: We have found 15 mutations in NRAS in 15 tumour samples. Fourteen did not have mutations in KRAS. Codon 61 is the most frequently mutated. The percentages obtained in our study are in agreement with previous studies.
Conclusion: This study demonstrates that a percentage of KRAS-negative colorectal tumours has mutations in NRAS, and that this status would be related to the response of anti-EGFR therapy.

PS15.23
The most frequent monogenic disorders in Russian population identified by microarray "Ethnogene"

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Introduction: It is known that every person has 5-7 pathogenic mutations in genome (mostly in genes of autosomal-recessive disorders). In such cases carrier status does not lead to clinical manifestation however, if both parents have mutations in the same gene, the risk for offspring to develop disease is 25%. In our clinical practice we prophylactically detect carrier status in spouses using genetic screening "Ethnogene" (APEX microarray technology). This screening program can be also used for diagnostic purpose. It is especially important at pre-clinical stage of disorder when there is a chance to prevent disease complications.
Materials and Methods: Microarray "Ethnogene" allows detecting the mutations leading to 60 monogenic disorders most frequent in Russia. In this study we prophylactically analyzed 336 persons including adults and children of different age.
Results: 8.6% of 336 genotyped patients were diagnosed to have monogenic disease (table 1). 70% of examined persons appeared to have 1-3 mutations in carrier status.
Conclusion: Application of microarray screening "Ethnogene" helps to reveal carrier status among couples planning pregnancy and to determine risk for offspring to be affected by certain monogenic disorders. Detection of monogenic diseases in yet asymptomatic patients allows preventing a number of complications by prescribing pathogenic therapy.

N=336 Disease	Gene	Mutation	Genotype	Cases	Frequency, %
Ichthyosis vulgaris	*FLG*	c.1501C>T (Arg501ter)	C/T (Arg/ter)	3	4,5%
		c.2282del4	N/Del	11	
		Compound heterozygous state for c.1501C>T (Arg501ter) and c.2282del4	Arg/ter; N/Del	1	
Hemochromatosis, type 1	*HFE*	c.187C>G (His63Asp)	G/G (Asp/Asp)	6	3%
		Compound heterozygous state for His63Asp and Cys282Tyr	His/Asp; Cys/Tyr	4	
Leber optic atrophy	*MTND4*	m.11778G>A (Arg340His)	G (Arg)	1	0,6%
	MTND6	m.14459G>A (Ala72Val)	G (Ala)	1	
Muscular dystrophy, limb-girdle, type 2A	*CAPN3*	.550delA (Thr184fs)	Del/Del (fs/fs)	1	0,3%
Familial Mediterranean fever	*MEFV*	c.442G>C (Glu148Gln)	C/C (Gln/Gln)	1	0,3%
Alpha-1-antitrypsin deficiency	*SERPINA1*	c.1096G>A (Glu342Lys)	A/A (Glu/Glu)	1	0,3%
Myotonia congenita, recessive	*CLCN1*	c.568GG>TC (Gly190Ser)	GG/TC (Gly/Ser)	1	0,3%
Total amount of patients				**29**	**8.6%**

PM15.24

Targeted Next-Generation Sequencing (NGS) Of Nine Candidate Genes With Custom Ampliseq In Patients And A Cardiomyopathy Risk Group

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Hypertrophic cardiomyopathy is a common genetic cardiac disease. Prevention and early diagnosis of this disease are very important. Because of the large number of causative genes and the high rate of mutations involved in the pathogenesis of this disease, traditional methods of early diagnosis are ineffective. We developed a custom AmpliSeq panel for NGS sequencing of the coding sequences of ACTC1, MYBPC3, MYH7, MYL2, MYL3, TNNI3, TNNT2, TPM1, and CASQ2. A genetic analysis of student cohorts (with and without cardiomyopathy risk in their medical histories) and patients with cardiomyopathies was performed. For the statistical and bioinformatics analysis, Polyphen2, SIFT, SnpSift and PLINK software were used. To select genetic markers in the patients with cardiomyopathy and in the students of the high risk group, four additive models were applied. Our AmpliSeq custom panel allowed us to efficiently explore targeted sequences. Based on the score analysis, we detected three substitutions in the MYBPC3 and CASQ2 genes and six combinations between loci in the MYBPC3, MYH7 and CASQ2 genes that were responsible for cardiomyopathy risk in our cohorts. We also detected substitutions in the TNNT2 gene that can be considered as protective against cardiomyopathy. We used NGS with AmpliSeq libraries and Ion PGM sequencing to develop improved predictive information for patients at risk of cardiomyopathy.

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PS15.25

The NR3C1 gene polymorphism in polish IBD patients undergoing glucocorticoids therapy

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Glucocorticoids (GCs) are still drugs of first choice in patients with exacerbation of inflammatory bowel diseases (IBD), although epidemiological studies show, that over 15% of patients would not respond properly to these agents. The glucocorticoid receptor, encoded by NR3C1 gene, is playing crucial role in the effects of GCs. A few of described polymorphism are functionally relevant. The aim of this study was to determine the essential loci: rs6189/rs6190 (p.ER22/23EK), rs56149945 (p.N363S), rs41423247 (BclI, IVS2+646C>G) of the NR3C1 gene and to correlate the obtained genotypes with the observed GCs therapy reaction.

Materials and Method: We analyzed DNA samples from 40 clinically diagnosed IBD patients treated with methylprednisolone and hydrocortisone. Patients were qualified to one of three groups - steroid resistant (6), steroid dependent (8) and steroid sensitive (26). The genotypes were determined using sequencing (p.ER22/23EK, p.N363S) and RFLP analysis (BclI).

Results: The p.E23K and p.N363S variants are not present in steroid resistant and dependent groups compared to 7.69% among steroid sensitive group. The allel G in BclI position was identified with frequency of 71.15 % in steroid sensitive group compared to 68.75 % in steroid dependent and 58.33% in steroid resistance. BclI carriers shown the significant increased sensitivity for GCs compared to rest of investigated patients (OR=0.103, 95% CI=0.008-1.282, p=0.04944).

Conclusions: Our initial studies indicate, that BclI polymorphism could be a useful molecular marker to identify polish IBD patients responsive to GC treatment.

PM15.26

Targeted next generation sequencing to unveil genetic markers of paclitaxel-induced peripheral neuropathy

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Introduction: Paclitaxel is a cytotoxic agent widely used in oncology. However, it frequently causes peripheral sensory neuropathy that can seriously impact patients' quality of life. Previous studies suggest an important contribution of genetic factors to the variation in paclitaxel neuropathy susceptibility; however, most part remains unexplained. In this study we aimed to identify genetic markers predictive of paclitaxel-induced neuropathy through candidate gene sequencing.

Materials and Methods: From a series of 380 breast and ovarian cancer patients treated with first-line paclitaxel, 170 cases (73 with low and 97 with high neuropathy) were selected for sequencing. A custom panel including 40 genes involved in paclitaxel pharmacokinetics and pharmacodynamics, and hereditary neuropathies was designed (TruSeq Custom Amplicon, Illumina). Libraries derived from patients' blood were sequenced in the MiSeq system (Illumina).

Results: The analysis pipeline includes selection of high-quality loss-of-function and missense variants and an analysis following two different approaches. First, for common variants a Chi^2 test to compare the number of alternative alleles among the two groups of patients. Second, a variance-component test, aimed to study low frequency variants in a gene basis. We found loss-of-function variants in *DHTKD1*, *EPHA5* and *EPHA8* genes in the high neuropathy group, and a preliminary analysis suggested that rare missense variants were overrepresented in *EPHA6* in the high neuropathy group. Full analysis will be presented in the conference.

Conclusions: Sequencing of candidate genes in well characterized series of patients seems a promising approach to unveil genetic variants associated with complex traits such as paclitaxel-induced neuropathy.

PS15.27

ICLDC Repository- a research platform for development of personalised medicine

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ICLDC Repository is a unique initiative for the storage and management of biological samples linked to clinical data, led by and integrated into Imperial College London Diabetes Centre (ICLDC), Abu Dhabi. The general purpose of the repository is to set up a resource that can support a diverse range of research intended to improve prevention, diagnosis and treatment of illness,

and promotion of health throughout the UAE society. The added benefits of the repository are reflected in the collection of samples and data for genetic analysis. Studying the genetic variation of the UAE population will enable the individualization of healthcare and contribute to disease identification, tailoring therapies and disease management strategies specific for this region.
ICLDC Repository undoubtedly represents a uniquely rich resource for investigating the relevance of a wide range of exposures to various health-related outcomes. More specifically, research conducted using ICLDC Repository genetic samples will facilitate the study of genetic variation that influence individual response to drugs.
ICLDC Repository is a newly developed concept in the UAE, with undeniable scientific value that can be extracted from such facility. The necessity for a better genetic understanding of the Emirati population is growing by the day and facilities such as ICLDC Repository will prove to be vital in accomplishing such an endeavor.

PM15.28
Combined Evaluation Of Genotype And Phenotype Of Thiopurine S-Methyl Transferases (TPMT) As A Profit Tool In The Clinic Management Of Patients In Chronic Therapy With Azathioprine

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Occurrence of adverse events (ADR) often occur during treatment with azathioprine (AZA) in patients with chronic autoimmune diseases. The response to AZA is influenced by the activity of thiopurine s-methyl transferases enzyme (TPMT): a low activity leads to accumulation of toxic metabolites, a high activity results in a higher production of methylated metabolite and therefore a lower therapeutic efficacy. To date 3 TPMT gene polymorphisms are associated with reduced enzyme function: 238G/C, 460G/A, 719A/G. Response to AZA can be predicted genetically with the study of polymorphysms and biochemically with the study of the enzyme activity. Integrated evaluation of TPMT genotype/phenotype is a useful tool in the clinical management of patients receiving AZA preventing ADR and/or side effects.
223 patients afferent to Medical Genetics of Niguarda Ca' Granda Hospital (Milan), were genetically analyzed for the 3 TPMT gene polymorphisms. The enzymatic TPMT activity was evaluated with HPLC assay.
199 patients resulted wild type (wt) and have tolerated therapy, 12 were found to be mutated and do not use AZA therapy, 12 patients resulted wt, but have developed ADR.
Genetic analysis of TPMT gene can predict the occurrence of ADR related to treatment with AZA predetermining TPMT activity levels; this text is not influenced by pharmacological and intra-individual variables. Conversely, genetic analysis focus only on three variables explaining about 80% of the altered TPMT activity. The biochemical test predicts dose-dependent ADR but the enzymatic assay suffers from pharmacological and/or individual variables. An integrated genotype/phenotype assessment of TPMT is a useful tool in the clinical management of patients receiving AZA for preventing ADR.

PS15.29
ePGA: an integrated electronic Pharmacogenomics Assistant for Personalized Medicine

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Introduction: Research and Direct to Consumer services increase the flow of genetic data. Interpretation of these data into meaningful information that could lead to clinical applications, requires the development of novel (bio) informatics tools and services. Here, we present an integrated electronic Pharmacogenomics Assistant (ePGA) that provides personalized genotype-to-phenotype translation services, linked to drug recommendations.
Materials and methods: ePGA is a web-service, which combines data from heterogeneous data sources (PharmGKB, dbSNP, Ensembl) and links them to available, up to date, clinical guidelines. ePGA users can query for combinations of genes, drugs and alleles, and browse related clinical guidelines. Moreover, users may upload genotypes in Variant Call Format (VCF) and receive personalized drug recommendations.
Results: To demonstrate ePGA we explored genome data from phase-I 1000 Genomes Project (1kG). Statistical analysis indicates that pharmacogenomics (PGx) profiles differ significantly among 1kG populations in most (~75%) of the studied pharmacogenes. In general, individuals of African ancestry exhibit greater PGx profile variation in most genes among populations, which can be attributed to increased genetic heterogeneity of African population.
Conclusions: The novelty of ePGA rests in its ability to translate genotypes into PGx phenotypes and drug recommendations, based on state-of-the-art pharmacogenomics knowledge. ePGA's acts as a "one stop shop" web portal for clinicians - by supporting them in making informed decisions, and for researchers - by providing a single place with information to understand, document and assess individuals' differences in drug efficacy.
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PM15.30
Modification of lipid spectrum due to pharmacogenetic interaction of prenatal retinoic acid administration with 7-gene-segment of rat chromosome 8

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Introduction: Polydactylous rat strain (PD/Cub) and spontaneously hypertensive rat (SHR) are two established rodent models of human metabolic syndrome. In PD/Cub predispositions for metabolic syndrome and polydactyly-luxate syndrome colocalize on the rat chromosome 8. Recently we have derived the minimal congenic SHR.PD-(D8Rat42-D8Arb23)/Cub (SHR-Lx) strain carrying only 7 genes of PD origin on SHR background.
Material and methods: Rat dams of SHR and minimal congenic SHR-Lx strains were treated with retinoic acid (atRA, 1 mg/kg at 13 ED) or vehicle. We then contrasted metabolic profiles (incl. oral glucose tolerance test (OGTT) and triglyceride and cholesterol in 20 lipoprotein fractions) of adult male SHR and SHR-Lx offspring under conditions of high sucrose diet with or without prenatal exposition to atRA.
Results: We observed differences in effect of atRA between SHR and SHR-Lx strains reflected by significant two-way ANOVA strain * atRA interactions (S*atRA). SHR-Lx PD5 displayed greater sensitivity to RA-induced metabolic dysregulations compared to SHR, including impairment of glucose tolerance (e.g. S*atRA $p = 0.04$ for glucose at 60 min. of OGTT), a shift towards less favorable distribution of cholesterol and triglycerides into the lipoprotein fractions and differences in size of cholesterol particles (e.g. S*atRA $p = 0.005$ for LDL particle size and $p = 0.004$ for HDL particle size).
Conclusions: We demonstrated that pharmacogenetic interaction of prenatally administered retinoic acid with a 7- gene region of rat chromosome 8 affects the distribution of cholesterol and triglycerides into the lipoprotein fractions along with other features of metabolic syndrome.
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PS15.31
Variants in clinical relevant genes among 2628 participants of the Dutch Rotterdam Study

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Within our Dutch population-based Rotterdam Study we have sequenced the exome of 2628 individuals. In this dataset we have made an inventory of variants present in the by Green et al. previously published 56 „actionable genes" that are important for and used in the clinical diagnosis of classical Mendelian genetic diseases.
We have selected truncating (stopgains, splice, frameshift) and nonsynonymous damaging variants (as predicted by a total of 7-8 prediction tools) and grouped them into 3 different tiers based on their predicted deleteriousness. Tier1 are variants classified as pathogenic or probably pathogenic by the ClinVar database (40 variants); Tier2 are variants classified as untested or probably non-pathogenic by the ClinVar database but annotated as truncating or loss of function variants (as predicted by 7-8 damaging prediction tools) (27 variants with 15 unreported in dbSNP138) and Tier3 are nonsynonymous and indel variants without a known clinical classification but nevertheless predicted to be truncating or damaging (by 7-8 damaging prediction tools) (30 variants with 18 unreported in dbSNP138). Initial analysis shows i.e. 2 novel variants (4 and 8 allele counts) predicted to be damaging in MSH2.
As participants in the Rotterdam Study are extensively phenotyped, novel variants from disease related „actionable genes" will be analyzed in relation to relevant phenotypes. Furthermore we are testing the presence of known clinical pathogenic variants in our exome data. This will add to the discussion on how to handle these kind of findings in population based studies, especially on whether or not to report back these findings to participants in the study and under which conditions.

PM15.32

Gene expression analysis of clozapine treatment in whole blood of patients with treatment resistant schizophrenia

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Objectives:
Clozapine is an atypical antipsychotic with a unique effect in treatment-resistant schizophrenia (TRS). We tested the effect of clozapine versus other drug treatments on peripheral blood gene expression in a sample of schizophrenia patients from the United Kingdom.
Methods: Blood samples from individuals receiving treatment for established schizophrenia were analysed for gene expression using on Illumina HumanHT-12.v4 BeadChips. After standard quality control procedures, 152 samples remained, including 55 from individuals receiving clozapine. Weighted Gene Correlation Network Analysis (WGCNA) was used to identify modules of co-expressed genes. The influence of the mood-stabilisers, lithium carbonate/ lithium citrate and sodium valproate, was studied to identify their possible roles as confounders.
Results
No significant change in gene expression was found for clozapine versus other antipsychotic medication at the individual gene or network level. Sensitivity analyses for lithium demonstrated an association with one gene co-expression module at the network level, and thus this was corrected for in the clozapine analyses.
Conclusions
Overall, this study finds no significant distinction between the effects of clozapine on gene expression in human whole blood samples versus that of other antipsychotic drugs at the individual gene or the network level. This study had limited power due to a small sample size and use of non-primary tissue but does
suggest that clozapine has similar effects on blood gene expression as other common antipsychotic treatments for schizophrenia.

PS15.33

Association of polymorphisms of genes involved in metabolism of oseltamivir with adverse drug reactions in a Mexican population

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Oseltamivir is a neuraminidase inhibitor extensively used in influenza outbreaks to prevent the release of progeny virions and thereby limit the spread of infection. There are reports about deaths and neuropsychiatric events in other populations with the use of oseltamivir, suggesting that this drug could inhibit also to human sialidases in a similar way that virus. Oseltamivir phosphate is a prodrug that is absorbed by Peptide transporter PEPT1 and effluxed by P-glycoprotein. Once inside of the cell, oseltavimir phosphate is converted by human carboxilesterase-1 to oseltamivir carboxilate, which is the active form to inhibit influenza virus neuraminidase. However, oseltamivir also has an inhibitory effect on human sialidases, which are important in various cellular functions including lysosomal catabolism. The aim of this study was to determine the single nucleotide polymorphisms (SNPs) for PEPT1(SLC15A1 gene), P-glycoprotein (ABCB1 gene), carboxilesterase-1 (CES1 gene) and sialidase (NEU2 gene) in >700 Mexican patients with oseltamivir therapy, and these data were correlated with side effects reported between 2010-2012. The SNPs evaluated were Gly185Val(rs1128501), Ser893Thr/Ala(rs2032582) and Ile1145Ile(rs1045642) for ABCB1 gene; Ser117Asn(rs2297322) for SLC15A1 gene; Gly143Glu and Arg199His (rs71647871 and rs2307243, respectively) for CES1 gene; and Arg41Gln(rs2233385) for NEU2 gene. Clinical data of patients were obtained from institutional electronic files. This work was approved by the Ethics Committee of Mexican Social Security Institute. We found that eight percent of patients showed side effects as depression, anxiety, seizures, and hallucinations among others. Genetic and allelic frequencies are presented and associated with the side effects of oseltamivir in a Mexican population. The authors thank CONACyT-Mexico for financial support (Grant number SALUD-2011-1-162243).

PM15.34

No impact of SLCO1B1 521T>C, and 388A>G polymorphisms on statin induced myopathy in the Hungarian population

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The first choice treatment of hypercholesterolemia and hyperlipidemia are the HMG-CoA reductase inhibitors (statins). One of the side effects of statins is the statin-induced myopathy (SIM) which occurs in 5-15% of patients. In the genetic background of SIM the *SLCO1B1* gene c. 521 T> C (p.Val174Ala, rs4149056) variation with the most significance association was described. The aim of our investigation was the analysis of the two commonly SIM associated *SLCO1B1* SNP's - c.521 T> C and c.388 A> G (p.Asn130Asp, rs2306283) - in Hungarian statin treated patients with SIM and without any side effects.
Sixty patients with SIM (male 23, female 37; mean age 64.3 ± 9.9 years) and 30 statin treated patients without SIM (male 11, female 19; mean age 53.2 ± 8.2 years) has been investigated. Specific TaqMan SNP Assays were used for the real-time PCR (ABI StepOnePlus System).
The allele frequency of the mutant C allele (rs4149056) was 81.7% in the SIM cohort (homozygous: 39 cases, heterozygous: 20 cases) and 81.6% in the group without SIM (homozygous: 19 cases, heterozygous: 11 cases). The allele frequency of the mutant G allele (rs2306283) was 45.8%, in the SIM group (homozygous: 9 cases, heterozygous: 37 cases) and 40% in the group without SIM (homozygous: 5 cases, heterozygous: 14 cases).
The presence of rs4149056 and rs2306283 major variants of *SLCO1B1* gene did not show any significant association to SIM in Hungarian patients. Analyses of larger cohorts, and SNP's of further genes, like *KIF6, COQ2 ATP2B1* are in progress.
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PS15.35

P53 tumor suppressor gene codon 72 polymorphism in patients with oral lichen planus - a pilot study

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Oral lichen planus (OLP) is a T-cell mediated autoimmune disease in which autocytotoxic CD8+ T cells (CTLs) trigger apoptosis of oral epithelial cells. Activated CTLs can produce Fas ligand and by binding to Fas lead to apoptosis. This Fas pathway and the action of p53 tumour suppressor gene are important in producing apoptosis. Current data demonstrate a link between these two factors at the transcriptional level. TP53 encodes a tumor suppressor protein, which plays multiple roles in apoptosis, cell-cycle control, and DNA repair. The TP53 codon 72 polymorphism produces variant with G/C, and the variant lead to an amino acid change in the protein product (Arg→Pro). The objective of this study was to assess the association of the TP53 codon 72 polymorphism with OLP. 93 Russian patients of European descent with OLP and 163 healthy donors were studied. Genotyping was performed by RT-PCR. The frequencies of TP53 C allele (P = 0.0008) and CC genotype (P = 0.004) in patients with erosive OLP were significantly greater than the corresponding values in the control group. The OR (95% CI) of erosive type of OLP cases with C as susceptibility allele was 2.25(1.38-3.64). Our study suggests that PRO at SNP P53 codon 72 is one of the genetic risk factors for OLP and that this polymorphism may be useful as one of the genetic markers for predicting the occurrence of this disease. Moreover, our data may provide genetic evidence to support the importance of P53 protein in OLP development. This research was funded by the RFBR grants 13-04-01489 and 14-04-97026.

PM15.36

Rationale for pretreatment genetic testing for VKORC1*2 polymorphism in vulnerable patients groups

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Vitamin K epoxide reductase complex, subunit 1 (VKORC1) has major role of vitamin K pathway in metabolic of warfarin (anticoagulation drug). Genotyping of VKORC1 most common polymorphism VKORC1*2 (rs9923231) is currently used in pharmacogenomics personalized therapy for warfarin treatment since it is linked to possibly serious adverse events in treated patients.

The main aim of this experiment was to assess the variation of VKORC1 in two typical subsets of patients subjected to anticoagulant treatment in order to estimate the actual need for genotyping prior to anticoagulant (warfarin) treatment.
First patient cohort was consisted of patients that had at least one thrombo-embolitic episode in their lifetime; second patient cohort was consisted of women that had recurrent spontaneous abortions. Healthy volunteers which did not have any thrombo-embolitic episode or family history of thromboembolism represented third cohort. VKORC1*2 polymorphism was genotyped by ARMS PCR method. Statistic tests used for genetic association analysis were: test of genetic association, adjusted for smaller sample size – Fisher Exact test (CI 95%); allele and genotype frequencies comparison among smaller subsets-exact P-value; odds ratio and relative risk ratio.
As a result of this experiment, no statistically significant allele association for VKORC1 with either investigated disorder was observed (p>0,05). Also, there was no observable difference in allelic distribution of VKORC1 as marker between thromboembolism-group and general population. Main conclusions are that in this retrospective analysis, we were unable to confirm that pretreatment genetic testing for VKORC1 polymorphism is justified in addition to standard precautionary measures related to anticoagulant treatment.

PS16.01
SeqPurge: highly-sensitive adapter trimming for paired-end short read data

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Trimming adapter sequences from short read data is a common preprocessing step in most DNA/RNA sequence analysis pipelines. For amplicon-based approaches, which are mostly used in clinical diagnostics, sensitive adapter trimming is of special importance. Untrimmed adapters can be located at same genomic position and can lead to spurious variant calls. Shotgun approaches are more robust towards adapter contamination. Untrimmed adapters are randomly distributed over the target region which reduces the probability of spurious variant calls.
When performing paired-end sequencing, the overlap between forward and reverse read can be used to identify excess adapter sequences. This is exploited by several published adapter trimming tools. However, in our evaluations on amplicon-based paired-end data we found that these tools fail to remove all adapter sequences and that adapter contamination can even cause spurious variant calls.
Here we present SeqPurge, a highly-sensitive adapter trimmer that uses a probabilistic approach to detect the overlap between forward and reverse reads of paired-end Illumina sequencing data. The overlap information is then used to remove adapter sequences - even if only one base long. Compared to other adapter trimmers specifically designed for paired-end data, we found that SeqPurge achieves a higher sensitivity. The number of remaining adapters after trimming is reduced by 40-75%, depending on the compared tool. The specificity of SeqPurge is comparable to that of the compared other tools. In addition to adapter trimming, SeqPurge can also perform trimming based on quality and based on no-call (N) stretches.

PM16.02
Gene expression profiles in generalized aggressive periodontitis

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Introduction: Aggressive periodontitis (AgP) is an inflammatory periodontal disease which is complex, multifactorial and destructive.High susceptibility for periodontal destruction and the relationship between inflammatory changes, and genetic factors remain unclear.In this study, we investigated molecular biomarkers which play role in the development of generalized aggressive periodontitis (GAgP) using gingival tissue samples through omics-based whole-genome transcriptomic while using healthy individuals as background controls.
Materials and Methods: Gingival tissue biopsies from 23 patients with GAgP and 25 healthy individuals were analyzed using gene expression microarrays with network and pathway analyses to identify gene expression patterns. To substantiate the results of the microarray studies, Q-RT-PCR was performed to assess the mRNA expression of MZB1 and DSC1.
Results: As a result of gene expression microarray studies, 5 significant gene networks were identified. The most up-regulated genes were found as MZB1, TNFRSF17, PNOC, FCRL5, LAX1, BMS1P20, IGLL5, MMP7, SPAG4, MEI1; the most down-regulated genes were found as LOR, LAMB4, AADACL2, MAPT, ARG1, NPR3, AADAC, DSC1, LRRC4, CHP2.
Functions of the identified genes that involved in gene networks were cellular development, cell growth and proliferation, cellular movement, cell-cell signaling and interaction, humoral immune response, protein synthesis, cell death and survival, cell population and organization, organismal injury and abnormalities, molecular transport, small molecule biochemistry.
The microarrays and real-time PCR resulted in similar gene expression changes, confirming the reliability of our microarray results at mRNA level.
Conclusion: Gingival tissue transcriptomes provide a valuable scientific tool for further hypothesis-driven studies of the pathobiology of periodontitis.

PS16.03
The effect of genetic variability on gene expression in human brain using whole transcriptome sequencing

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Expression quantitative trait locus (eQTL) analysis allows the identification of common DNA variants that regulate gene expression.
As part of the UKBEC project, we performed eQTL analysis on whole transcriptome RNA sequencing (RNA-seq) data from 134 neuropathologically normal post-mortem human brains (180 samples from two separate regions: putamen and substantia nigra). After standard quality control steps, the RNA-seq data were combined with SNP genotype data from the Illumina Omni-1M and Immunochip arrays and then imputed to the 1000-Genomes resource, before genome-by-transcriptome association analysis using the MatrixEQTL package.
We identified eQTLs at both the whole-gene and exon levels. We detected 1883 independent eQTL signals in putamen and 1072 in substantia nigra at the whole-gene level and 3523, 1848 respectively at the exon level. We found strong evidence for region-specific eQTLs both at the gene and exon-level . We also report high overlap (73%,47%) with our previous eQTL study using microarray data. Finally, we compared our eQTL signals with signals obtained for common, consistent Allele Specific Expression (ASE) signals. We found evidence for (1) ASE signals that converted to eQTL signals in a „chromosome-independent" manner; (2) ASE signals that converted to eQTL signals but in a manner suggestive of chromosome-dependence; (3) consistent ASE signals which did not convert into eQTL signals. These patterns reveal the complexity of genetic regulation as reflected in eQTL and ASE signals.
Our dataset provides a valuable resource for the neuroscience community and all results will be made publicly available through our portal (www.braineac.org).

PM16.04
HumanMine: An integrated data resource for Human Genomics and Proteomics.

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HumanMine (www.humanmine.org) is a new integrated database of human genomics and proteomics data built using the InterMine data warehouse system. Datasets incorporated include SNPs, GWAS studies, pathway and interaction data, protein localisation and expression studies, disease and phenotype data as well as genome annotation and sequences with plans to increase utility in disease studies.
HumanMine is designed for integrative analysis and can be accessed through a user-friendly web interface. For bioinformaticians, extensive web services as well as programming interfaces for most common scripting languages support access to all features.
The web interface includes a useful identifier look-up system, and both simple and sophisticated search options. Interactive results tables enable exploration, and data can be filtered, summarised, browsed and exported as well as transmitted for external analysis in Galaxy and GenomeSpace. A set of graphical analysis tools provide a rich environment for data exploration including statistical enrichment of sets of genes or other entities.

HumanMine complements other InterMine-based data analysis platforms that are available for mouse (www.mousemine.org), rat (ratmine.mcw.edu), budding yeast (yeastmine.yeastgenome.org), plants (www.araport.org/thalemine), nematode (www.wormbase.org/tools/wormmine), fly (www.flymine.org) and zebrafish (www.zebrafishmine.org). Through InterMine we aim to make it easier for users to navigate between databases, and facilitate cross-organism analysis. All of the above InterMine databases are freely available resources run by members of the academic research community.
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PS16.05
Bayesian methods for assessing shared genetic effects in auto-immune disease

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Genome-wide association studies (GWAS) have linked many single nucleotide polymorphisms (SNPs) to many phenotypes. Often, one SNP will show associations with multiple diseases, which may point to a shared genetic architecture, and thus common underlying etiological pathways. New methods are needed to help formally detect and assess pleiotropy in a way that can be applied genome-wide. We have developed an approach using Bayesian inference and applied it to published data on the evidence of association at 107 SNPs, in 7 different auto-immune diseases, published by Cotsapas et al. Our method makes use of readily available summary statistics of genetics association, and we use simulations to explore power to detect pleiotropic associations. As the approach is based on summary statistics we can easily obtain a probabilistic assessment of each of the possible 2^7 models of pleiotropy. The complete posterior probability distribution not only allows us to determine the „best" model, but also to see by how much it improves on the null model of no association with any phenotypes, as well as all other possible models. We highlight its use in helping assess the genetic similarity between disease phenotypes.

PM16.06
Single molecule BCR-ABL1 fusion transcript sequencing in routine screening of CML patients

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By long-read Pacific Biosciences (PacBio) sequencing we are developing workflow for detection of BCR-ABL1 tyrosine kinase inhibitor (TKI) resistance mutations in patients with chronic myeloid leukemia (CML). Our assay enables rapid sequencing of a ~1.6kb BCR-ABL1 cDNA amplicon without the need of a nested PCR. This results in a collection of highly accurate full-length sequences that represent the diversity of BCR-ABL1 molecules in a given sample. Mutations down to a level of 0.5% are clearly detected using this approach. Moreover, the long PacBio reads makes it possible to resolve the mutational composition of all different clones present in CML patient samples. Since compound mutations might confer cross-resistance to multiple TKIs, the information provided by our assay can directly influence the choice of therapy. Our results also show the presence of multiple splice isoforms of BCR-ABL1 in several of the samples. However, we have at present no indications that these splice isoforms play any role in the development of TKI resistance.
To evaluate if our method is ready for introduction into clinical routine we are now conducting a pilot project, where CML patient samples screened by Sanger sequencing are analysed in parallel with the PacBio assay. A streamlined workflow has been developed both for the sample preparations and bioinformatic analyses. Also, a web based reporting system has been constructed to communicate mutation results to the clinicians. Our preliminary results show that the PacBio workflow gives a fast turn around time and a higher sensitivity for mutation screening as compared to Sanger sequencing.

PS16.07
TidyVar: fast and accurate variant caller

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We present a novel approach to calling variants (SNPs and short indels) in DNA sequencing data from multiple individuals. The new algorithm is fundamentally different form the existing methods. It uses string matching and pattern recognition to discover and genotype variant alleles. The algorithm implementation (TidyVar) outperforms the current state of the art variant calling software (GATK) in accuracy and speed. In particular it excels in discovery and genotyping of insertions and deletions. Moreover unlike GATK and other Bayesian variant callers TidyVar can call variants in small targeted genome regions. We describe the basic principles of the new algorithm and compare the variant calls by TidyVar and GATK on an example dataset.

PM16.08
Detection of new genomic regions related to celiac disease based on genetic ancestry

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Celiac disease, an autoimmune disease that is developed in presence of gliadine, has a complex genetic basis. The most important genetic factors are located in the HLA region, explaining the 40% of the genetic component. In addition, various genomic regions that could be related to the disease have been identified but these regions could vary among populations.
With the aim of finding unknown genomic regions related to celiac disease, we designed a new approach based on the ancestry of subpopulations and used it to reanalyze the data from a case/control study of celiac disease genotyped using the Immunochip platform. First, we defined blocks of haplotypes to choose unrelated SNPs, in total 8537 blocks. Then, we analyzed the ancestry of individuals to define subpopulations and the optimal number of ancestries was 30. Finally, each subpopulation was analyzed using association analyses.
The results of association analysis in each subpopulation were slightly different. Although the HLA region was the most significant, the rest of genomic regions that could be related to the disease were mostly different in each subpopulation. 370 out 381 SNPs with a p-value<10-4 were located in genomic regions that were not previously related to celiac disease. Thus, the approach we developed could be useful to detect genomic regions that were not previously known to be related to celiac disease and to determine new genomic components that modulate risk to celiac disease.

PS16.09
BioExpress: Cloud service for massive bio data using hybrid cluster which can run Hadoop and general Linux programs

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Owing to the exponential growth of biological data since the introduction of next generation sequencing technology, analysis of massive bio data became a more complicated and difficult problem. To find out meaningful information from these massive data, researchers need IT skills to compose and run complicated bioinformatics analysis process which is constituted many opensource programs. IT infra such as computing servers, network devices, and storage is also essential to run pipelines. Consequently small research groups without IT facility have a big problem to analyze massive bio data. The research groups with IT facility have also a difficulty in maintenance of IT facility. To address this problem, we developed cloud service for massive bio data (BioExpress). BioExpress consists of two modules: OpenBio and Closha. OpenBio is hybrid cluster system which can run Hadoop and Linux programs. It is based on HDFS which can run Hadoop programs and disk caching, the technique which transfers a file in HDFS into general Linux file system in each request, enables the executing of Linux program. It is economic than general cluster system with shared storage because it is based on HDFS which is cheaper than shared storage. BioExpress has also parallel processing feature using MapReduce in Hadoop. Closha is an automatic workflow modeling system that researchers can represent the process of bio-data analysis as a workflow which is composed of a sequence of analysis tools by connecting the output of preceding tool and the input of following tool in sequence, with same formats. Users can easily analysis the complicated and massive bio-data through Closha.

PM16.10
CNV calling and association with body mass index in adults: benchmarking and meta-analysis.

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Many SNPs influence common disease susceptibility, but explain only part of the heritability (<3% for BMI). We have shown that the rare variants, like the 16p11.2 rearrangement, can have substantial impact on BMI. To search for more such examples genome-wide, we use CNV calls from genotyping arrays of very large number of cohorts in order to identify new rare CNVs associated with BMI.
As genechip-based CNV calling has high false positive rate, we first developed a novel quality score (QS) to estimate the probability of a CNV call being true. To derive this QS we called CNVs for five cohorts (N=6'628) using PennCNV, QuantiSNP and CNVpartition. A CNV is considered to be true if at least 70% of its length is detected by all software. To predict which PennCNV calls are true, we built a predictive model using different CNV- and sample-related quality metrics. We termed this continuous predictor of true CNV status as QS. We simulated traits associated with true CNV status and ran associations between these traits and PennCNV calls. Results showed that our QS-based association can yield up to 5–fold increase in power compared to classical filtering approaches, in particular for low frequency (<10%) and low quality CNV calls.
Using our QS-based association we performed a pilot CNV association for BMI (N=4'381). First results show trends of association for a CNV located 277kb upstream of *SH2B1* (P-value=0.00336). In collaboration with the GIANT consortium we expect to scale up to 100'000 samples within the next four months.
ZK received financial support from the SNSF (31003A-143914, 51RTP0_151019).

PS16.11

OneSeq enables simultaneous detection of genome-wide copy-number-changes, cnLOH, indels, and low frequency multi allelic gene mutations

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Agilent's OneSeq is a revolutionary all-in-one SureSelect target enrichment assay with accompanying SureCall software that detects genome-wide copy-number-changes (CNCs), copy-neutral loss-of-heterozygosity (cnLOH), indels, and point mutations. The assay comprises a set of backbone probes for CNV and cnLOH, plus user selectable mutation detection probes. Clinical researchers will benefit from the lower costs of a single OneSeq assay compared to using different assays to detect each type of variation. In particular, low-pass WGS cannot detect cnLOH; while focused target enrichment panels are not effective at measuring genome-wide CNCs.
OneSeq backbone probes are optimized to detect CNC and cnLOH by capturing high minor allele frequency SNPs. Specificity is ensured via consideration of Shannon-Weiner sequence complexity, DUST score, GC content and mappability. A novel algorithm has been developed and implemented in SureCall to best analyze OneSeq data. The algorithm streams sample and reference data to generate log-ratios of sequencing counts. Chromosomal break points are detected from the log-ratios using an un-decimated wavelet transform. False discovery rate (FDR) is controlled; the breakpoints passing the FDR control are scored for statistical significance. The aberrant intervals are then assigned copy numbers by examining the median values at different wavelet scales. In parallel, SNPs are called using the SNPPET algorithm, which facilitates calling of cnLOH.
OneSeq detects known aberrations of sizes as small as 150 kB, and 2 MB cnLOH, while allowing simultaneous measurement of indels, and point mutations. OneSeq offers a practical solution by converging genetic technologies while maintaining cost-effectiveness and throughput in one comprehensive assay.

PM16.12

How good is my run? Whole genome resequencing and Targeted panel sequencing enrichment coverage analysis

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Introduction: With the rapid advancement of Next Generation Sequencing (NGS) and the parallel development of bioinformatics tools to analyse the data whole genome re-sequencing (WGS) is now becoming feasible in medium sized labs. Furthermore, targeted panel sequencing (TPS) is already used as a diagnostic medium at many medical centres. We describe a versatile tool that provides coverage information for WGS and TPS runs to assess whether the sequencing data has covered the genome and all the targets sufficiently for downstream analysis including variant calling.
Materials and Methods: We bring together the forces of open source software including R, bedtools, samtools, and circos to provide coverage statistics and quality plots for assessing the quality of the run.
Results: For WGS we provide tables and plots describing among others: per chromosome coverage statistics, duplicate read percentage, repeat regions, segmental duplication, known copy number and structural variation coverage, translocations, average gene coverage and clinically actionable exon coverage.
For targeted panel the user defines the panel manifest, the targeted transcripts, the level of coverage required and optionally a set of expected targeted variants. The tool summarises the coverage in all targets focusing on the exons and the expected variants, reporting all regions not sufficiently covered as a first indication of the suitability of the run for clinical diagnosis.
Conclusions: As NGS moves from research to clinic, this tool for assessing the quality and coverage of a run can be an integral part of the analysis pipeline.

PS16.13

Genome-wide association study of 41 circulating cytokines

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Introduction**:** Cytokines have essential roles in regulation of immune response. Dysregulation of immune system have been linked to various autoimmune diseases such as inflammatory bowel diseases and rheumatoid arthritis. The purpose of this study is to shed light to yet unknown biology behind immune system regulation and pathogenetic mechanisms behind autoimmune diseases.
Materials and Methods: Both the genotype and cytokine data were available from three Finnish population based cohorts: The Cardiovascular Risk in Young Finns Study, Finrisk97 and Finrisk02. Total of 8153 subjects were included in this study. Cytokine measurements have been performed with Bio-Rad's premixed Bio-Plex Pro Human Cytokine 27-plex Assay and 21-plex Assay. The amount of bound cytokine was detected using streptavidin-phycoerythrin conjugate. 1000 genomes imputation panel was used to impute genotypes. Snptest software was used to conduct genome-wide scans. Meta-analyses were performed with METAL software. Following filters were applied to data: info > 0.7, minor allele count > 10 and association test info > 0.7. Cis eQTL analyses were performed for lead SNPs.
Results: We identified 14 new loci previously not associated with cytokines, hsCRP or white blood cell count. Six of these loci are located near a SNP previously associated with an autoimmune disease. Total of 29 loci had genome-wide significant association with the concentration of at least one cytokine.
Conclusions**:** This study have provided important insights to development of autoimmune diseases and identified cytokines that probably have important role in development of these diseases.
The study was supported by Juho Vainio Foundation.

PM16.14

SEDAN: A cloud enabled platform for analyzing and storing sequencing experiments within a clinical context

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The rapid advances of high-throughput sequencing technologies have been revolutionizing the way we study the human genome. To use and validate these new technologies in a clinical setting, a standardized analysis workflow is needed tailored to sensitivity, specificity, safety, and usability requirements.
Based on these prerequisites, we have been developing a cloud enabled sequence data analysis pipeline (SEDAN) covering the complete analysis workflow: data cleaning, read mapping, variant calling, annotation, and decision support. SEDAN has been designed as a multistep application supporting the execution of the whole pipeline or only specific parts of the workflow. In addition, it performs on the fly transformation of coordinates to different reference sequences (e.g., hg19 - Refseq).
The pipeline is centered on proven open source tools and is able to deal with

data generated by all widely-used sequencing platforms (NGS and Sanger). It provides predefined parameter sets and can be used without previous knowledge of informatics. Results are output as spreadsheets and in standardized formats. In addition, they are displayed in a graphically interactive way together with meta-information. The pipeline is tightly integrated into the web-based Platomics platform, providing data management capabilities, user authentication, and a graphical user interface. Each analysis run, including used parameters and result files, is automatically stored and can be easily queried and compared.
The analysis pipeline and platform are actively used in a clinical setting and have been rigorously validated. Together, they provide a straight forward and easy to use solution for analyzing sequencing data in a clinical context.

PS16.15
Orphanet: a database of genes with clinical significance in rare diseases

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Sequencing the whole human genome and the advances made in NGS techniques allow a number of genetic variants to be detected, although their functional impact is still largely unknown. To harness this information, it is necessary to characterise a complete list of human disease genes.
To contribute to this goal, Orphanet maintains a database of genes, loci and non-coding RNA of clinical significance in rare diseases (RD). Gene-disease relationships are described as: causative mutation (germinal/somatic, loss-of-function/gain-of-function), modifier gene, major susceptibility gene, gene playing a major role in the phenotype of a chromosomal anomaly. Candidate genes tested in the clinical setting are included. Genes are linked to a clinical, poly-hierarchical classification of RD, and aligned with OMIM, HGNC, UniProtKB, Genatlas, ensembl, Reactome and IUPHAR, allowing for interoperability with mutation databases. Data are captured from literature and databases surveys, manually curated and expert-assessed. They are visible on the Orphanet website (www.orpha.net), downloadable in Orphadata (www.orphadata.org) and in the Orphanet Rare Diseases Ontology -ORDO- (http://bioportal.bioontology.org/ontologies/ORDO). The database includes 3376 genes and is updated monthly.
The added-value of the Orphanet database of genes lies in the relationships provided to clinically defined conditions, which are unique whatever the number of genes involved and stable in time; in the possibility to cluster genes by disease groups, or RD by genes; and in its expert curation. The database can be used as a reference to compare new genetic information with state-of-art knowledge on RD genes.
This work is supported by „RD-Action", a Joint Action receiving funding from the European Union in the framework of the Health Programme.

PM16.16
Deciphering Developmental Disorders: Clinical review and reporting in practice

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The Deciphering Developmental Disorders study is on course to analyze the exomes of 12,000 children with developmental disorders, recruited from the 24 regional genetics services across the U.K. and Republic of Ireland. The DDD study is a collaboration between the Wellcome Trust Sanger Institute and the U.K. Department of Health and aims to research the causes of developmental disorders and to diagnose patients where standard genetic testing has proved unrevealing. Here I describe a software tool for converting genetic findings into a diagnosis as part of a population scale solution.
To achieve high-throughput the tool enables fully automatic rule based, and user driven delivery of reports, variants, evidence and comments to our collaborators in the regional genetics services. Both modes are required as not all genetic findings are relevant to the patient and complex cases must be manually reviewed.
To support efficient manual review users can set their own filters and prioritize patients for later sessions. The DDD study is also a research project so the tool is designed to support reinterpretation of patients if new variants are discovered or if the variant consequence or inheritance has changed.
To help interpret the variant the tool provides an integrated display of patient and family information as well as views of the raw data. The tool helps to create a valuable resource for research into variant prioritization by recording pathogenicity and relevance to the patients phenotype.

PS16.17
How to find the second patient

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In recent years disease causing genes were identified for many monogenic disorders in high-throughput sequencing studies for exomes and whole genomes. Usually such approaches were successful, if a case group with several phenotypically similar patients was available. The analysis of such cohorts was relatively simple from a statistical point of view and is often referred to as filtering by overlap.
However, for all the cases that did not yield conclusive results more sophisticated approaches are now required, that is finding similar patients and searching for significant gene associations. We designed an online platform that assists in matching patients on a phenotypic as well as on the genotypic level. This will result in a case groups for which we test whether one gene bears a significant burden of rare variants. If a significant association is detected all users who contributed a case to this phenotypic cohort will be informed so that they can join in working up the identified candidate mutations. We demonstrate use cases in which the status of variants of unknown clinical significance could effectively be resolved by a community driven effort.
Currently more than 1000 expert users are registered on the platform and participate in illucidating Mendelian disorders.

PM16.18
DIDA: the first digenic disease database

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Although there exist many human disease-related genetic variant databases (e.g. HGMD, SwissVar), no public effort has been made to organize these data so that one can investigate the oligo- and polygenic nature of their disease-of-interest. However, it has been shown that many disorders, classically considered as monogenic, may be better explained by more complex inheritance mechanisms. One example can be found in those diseases with imperfect genotype-phenotype correlations, which might, in a monogenic context, be considered as showing reduced penetrance, but could in fact also be explained by a digenic inheritance model. This example pinpoints the need to develop new databases and services focused on complex inheritance models.
Here we present DIDA (DIgenic DAtabase): a manually curated database collecting human digenic disease instances. DIDA was created by collecting all digenic disease data published in scientific literature until December 2014. We manually screened the literature to ensure the high quality of this ex novo digenic database. For every publication describing patients with a disease explained by a digenic inheritance model, we annotated causative variant-pairs and enriched every instance with different features. These features include variant- and gene information with genomic-, cDNA- and protein coordinates, information regarding the functional effects of the variants, disease name and OMIM-id, clinical symptoms with HPO-terms and a digenic effect category (influence on the presence, severity, age-of-onset or symptoms of the disease). As such, this database forms a basis for understanding how the interplay and weight of variants leads to disease, which in turn may provide novel insights into diseases classically considered as monogenic.

PS16.19
Integrating data sources in druggability analysis of genes implicated in dilated cardiomyopathy

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Introduction: Dilated cardiomyopathy (DCM) is characterized by systolic dysfunction and dilation of the left ventricle of the heart, with a 5-year mortality estimated in 50%, and is the main indication for heart transplantation. Current treatments primarily work by slowing down disease progression. Treatments targeting genetic factors may potentially prolong survival and reduce side effects. A literature search identified 110 genes implicated in DCM. We analyzed the druggability properties of these genes and related genes in pathways. A list of gene-drug interactions was generated based on bioinformatics techniques, and we aim at identifying drugs that can be repurposed for DCM treatment, using information on indications and side

effects of these drugs.
Materials and Methods: We identified 343 drugs that act on DCM-related genes, using DGIdb [1], chEMBL [2] and DrugBank [3]. We developed a Python script to identify synonyms of each drug, based on definitions from STITCH [4], and integrated these drugs with indications and side effects from SIDER [5].
Results: From the 343 drugs mapped for the genes of interest, 114 were found by the algorithm in the aliases database. The search returned 14589 synonyms for the original names, a list of side effects for each drug listed (10359 in total) as well as their indications (934 in total).
Conclusions: Integrating data sources is a necessary step towards more complex analyses that may lead to better and faster translation to health care.
REFERENCES
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PM16.20
Transcriptome analysis of mouse ES cells carrying a human chromosome 21

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Down syndrome is most likely the result of a gene dosage alteration. In order to further characterize this dosage imbalance, we investigated the transcriptional changes occurring in tranchromosomic mouse ES cells carrying an additional human chromosome 21 (HSA21) (Hernandez et al. 1999). By adding a whole freely segregating human chromosome, this model properly recapitulates the trisomy 21 conditions and offers a way to examine the expression of human genes in a mouse genomic context.
We used mRNA-sequencing to determine the transcriptome profile of these transchromosomic cells, using the parental wild-type mES cells as controls. Four replicates of each group were sequenced on the Illumina HiSeq. Reads were mapped with TopHat against a hybrid reference containing both the mouse genome and the HSA21 sequences.
A preliminary differential expression analysis (EdgeR) revealed 8'101 protein-coding genes dysregulated in the transchromosomic cells (Bonferroni<0.01), suggesting that the presence of a HSA21 can extensively disturb the mouse transcriptome.
Additionally, we compared the expression level (RPKM) of each HSA21 gene (present in 1 copy) and its corresponding ortholog in the mouse genome (2 copies). Interestingly, very few genes showed the expected 1:2 expression ratio between the 2 species. Whereas some genes were exclusively expressed from HSA21, others were on the contrary completely silenced. Further analyses will help to understand the regulatory network controlling the expression of these human genes in this unrelated mouse genomic context. The model will also contribute to the understanding of evolution of the regulatory landscape in mammals.

PS16.21
European Genome-phenome Archive (EGA): a secure archive for genomic and phenotypic data

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The European Genome-phenome Archive (EGA) facilitates the secure storage and controlled distribution of genetic and phenotypic data, for the purpose of biomedical research.
Our collections include major reference data for rare and common diseases, including data derived from the UK10K project, Blueprint and the International Cancer Genome Consortium (ICGC), as well as control sets for use in addition to the public reference panels, such as the 1000 Genomes project.
As of January 2015, the EGA securely stores ~1.6 petabytes of data derived from ~600K unique samples across ~1300 distributable datasets.
The EGA have implemented fundamental changes to facilitate data discovery and improve data dissemination to enhance the user experience of EGA users.
The new 'Data Mart' webpage enables a user to sort, filter and select files based on 'Study', 'Dataset' or 'Sample' centric criteria before initiating a download.
We have also introduced a data streaming service to disseminate data files. The service provides robust, reliable and flexible downloading capabilities, enabling the user to download files direct from the EGA website or using the command line.
The new services also enable the user to query all EGA public metadata through a REST API, as well as downloading metadata from the EGA website.
Future plans include providing the user with the facility to generate 'BAM slices' for viewing in a genome browser.
The EGA is maintained by European Bioinformatics Institute (EMBL-EBI) and the Center for Genomic regulation (CRG) and is available at both www.ebi.ac.uk/ega/ and https://ega.crg.eu/.

PM16.22
A tool for rapid aggregation of eQTLs in human cells

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While genetic variation in the coding regions of genes can drive disease, it has been shown that SNPs associated with complex disorders and some pharmacologic phenotypes are enriched for expression quantitative trait loci (eQTLs): polymorphisms that influence the expression of these genes. Despite the efforts of researchers, eQTLs that have been identified in existing studies are often „locked up" in the supplementary methods and difficult to obtain from the published material. Existing tools for browsing these SNPs are often time intensive and difficult to use. This issue is further complicated by the fact that data scientists and researchers looking for statistically significant relationships between eQTLs, genes and disorders often lack disease-centric knowledge that would be needed to identify the potentially important single nucleotide polymorphisms (SNPs). To this end, we have implemented a software tool that gives easy and unified access to eQTLs described in published studies and quickly arrive at a list of SNPs that could be relevant for a disease or gene. We have currently included results from four studies that have identified eQTLs in human cells: 1 based on blood-, 1 on intestine-, and 2 on liver-cells. By distributing the software as an IPython notebook, scientists can examine and extend the existing code to include data from other sources as well. Additionally, this approach simplifies the subsequent analysis, as the resulting SNP list can be further refined and explored using the extensive Python codebase for artificial intelligence, machine learning and other algorithms, resulting in rapid knowledge discovery.

PS16.23
A pipeline for eQTL-analysis using Illumina microarray data

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Throughout the last decade GWAS have identified numerous SNP-disease-associations. However, a majority of these SNPs are located in non-coding regions, which necessitates a comprehensive functional annotation. Here, expression quantitative trait loci (eQTL) analyses which enable the identification of SNPs that affect gene-expression in a tissue and context-dependent manner have gained major attention. Therefore, we aimed at establishing an automated workflow for identification of eQTLs from high-throughput genomic and transcriptomic data. Genome-wide genotype and expression data was generated using Illumina's Omni-Family and HumanHT-12 bead arrays. Subsequent imputation of genotypes (1000Genomes) ensures comparability of the eQTL-findings across studies and enables integration with GWAS data. Robustness of the workflow is guaranteed by application of different filter steps, i.e. removal of non-expressed, ambiguous and low-confidence transcripts from expression data and filtering of genotypes according to standard QC parameters. eQTLs are identified via linear regression using *MatrixEQTL*. Inclusion of covariates in the regression analysis ensures controlling for confounding effects in the data and leads to an improved and highly reliable identification of eQTLs. The pipeline was successfully applied to identify tissue-specific eQTLs from stomach, hair and hippocampus. Here, we found more than 10,000 eQTLs in the investigated tissues, ~35% being tissue-specific. The data has been used to functionally annotate GWAS data for Barrett's esophagus, male pattern baldness and psychiatric disorders. Thus, our pipeline presents a fast, standardized and easily applicable me-

thod for identification of functional genetic variance derived from microarray and RNAseq data and can help to elucidate biological mechanisms at known disease risk loci.

PM16.24
OpEx: an exome analysis pipeline optimised for sensitive and specific indel detection

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Exome sequencing is being used by increasing numbers of research and clinical laboratories. However, simple, user-friendly exome analytical pipelines are not readily available. In particular, detection of insertion/deletion (indel) variants has proved challenging in NGS data, particularly for rare indels. It is essential for research and clinical disease genetics that indels are robustly detected because of the prevalence of this variant class as a pathogenic mutational mechanism.
To enable rapid, automated and robust analysis of exome sequencing data optimised for indel detection, we created the OpEx pipeline. OpEx uses Stampy for alignment, Platypus for variant calling, CAVA for variant annotation and provides useful additional information such as coverage metrics. OpEx is easy to use, requiring a single command and input FASTQ files to return a tab-separated file containing clinically annotated substitution and indel variants in an individual. We evaluated OpEx performance with a set of orthogonal validation data, including 730 Sanger sequencing evaluations. Importantly, OpEx is both sensitive and specific for indels, with ~95% of positive and negative sites correctly identified, and a low false positive rate of 3%. OpEx is run independently at the sample level, providing similar performance for any number of samples and thus allowing analysis to keep pace with sequencing output. Due to its excellent performance and suitability for the clinical and lab settings, OpEx is our standard exome analysis pipeline, used to analyse over 12,000 exomes to date. We are now making the OpEx pipeline freely available for others to use (by April 2015).

PS16.25
Functional annotation of obesity SNPs underlying potential parent of origin effects

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Genome-Wide Association Studies (GWAS) were successfully applied to discover genetic variants associated with obesity, however some heritability were unexplained. Analyses of potential parent of origin effects (POE) may provide further insights into genetic mechanisms of obesity. The aim of study was to identify novel functional SNPs/regions which would contribute to obesity.
Genome-wide genotypes from Sorbs (N=525), a German self-contained population, were phased using AlphaImpute. Three different GWAS were applied in PLINK: (i) standard association, (ii) considering paternal and (iii) maternal alleles. Ten top tagging SNPs from paternal and maternal GWAS were selected respectively. An R package FunciSNP was used to identify correlated SNPs.
Totally,109 SNPs and 180 SNPs correlated with tagging SNPs underlying paternal and maternal POE respectively. Two transcription factors SF1 (steroidogenic factor 1) and LRH1 (liver receptor homolog-1) putatively bound at rs1204880 underlying paternal POE. SF1 involves in determining sex and differentiation while LRH1 affects bile acid metabolism and glucose homeostasis. Rs1204880 was highly correlated (r2 =1) to tagging SNP rs942459 and located within the putative promoter of PADI6 (Peptidyl Arginine Deiminase, Type VI) which may associate with reorganizing cytoskeletal in egg and during early embryo development. MicroRNA binding sites were identified at rs11180547 and rs4562666 underlying maternal POE.
Incorporating high-throughput epigenetic data, variants from 1000 Genomes and various genomic databases into POE specific GWAS may reveal novel putative SNPs located in potentially functional regions and maybe involve in obesity.
This work was supported by German Research Foundation, German Diabetes Association, DDS Foundation and IFB AdiposityDiseases.

PM16.26
Integrated Genome Mapping in Nanochannel Arrays and Sequencing for Better Human Genome Assembly and Structural Variation Detection

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De novo genome assemblies using purely short sequence reads are generally fragmented due to complexities such as repeats found in most genomes. These characteristics can hinder short-read assemblies and alignments, and that limits our ability to study genomes.
The BioNano Genomics Irys System linearizes long DNA molecules, thus yielding single-molecules containing long-range information. These hundreds of kilobases molecules can capture structural information that may be missed by other sequencing platforms. The assembled genome maps from these molecules can scaffold sequencing contigs to validate the accuracy of the sequences, and to anchor the adjacent sequences into the proper order and orientation. The long-range hybrid scaffolds can identify novel chromosomal rearrangements recalcitrant to short-read alignment or reference-guided assembly approaches.
We present a comprehensive analysis of a human genome by combining single molecule genome mapping with one of the most annotated sequence assemblies, the HuRef assembly. Overall, we found that the assemblies of two technologies correspond well, and the resulting hybrid scaffolds are highly contiguous, with a N50 of >35Mb, a value typically unachievable by short-read sequencing. In addition, we compared the structural variation with calls previously detected in the HuRef assembly, and found multiple novel variants spanning over hundreds of kilobases in size. Some of these variants reside in areas where the sequence assembly was poorly covered or was highly fragmented; yet these variants encompass numerous genes, and can be of functional importance. Finally, we identified genome maps that span over the remaining reference gaps, and maps that resolve and measure long tandem repeats.

PS16.27
Structural Variation Discovery by De Novo Assembly Using Extremely Long Single-Molecules in Human Disease and Non-Disease State Genomes

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Structural variation analysis (SVA) of human genomes is usually a reference based process and therefore biased and incomplete. In order to have a comprehensive analysis of structural variation, a de novo approach is needed. As a result of limitations of DNA sequencing methods, it is not feasible to create high quality de novo assemblies for detecting and interpreting structural variation that are refractory to high throughput or short-read technologies. Using a single molecule genome analysis system (Irys®), we produced high resolution genome maps that were assembled de novo, these maps preserve long-range structural information necessary for structural variation detection. Dozens of human genomes have been de novo assembled by Irys to date. Structural variation analysis reveals insertions, deletions, inversions and translocations. We have generated genome maps for two trios. From these genome maps, we detect hundreds of structural variants, including large deletions in genes in the mother and son from the AJ trio. We have also investigated the amylase locus in both trios as well as ~20 other individuals and have found at least 15 different structural variants. Human amylase genes have variable copy number and this variation is believed to have been evolved to adapt to increase starch intake. We were able to identify multiple copy neutral variants, i.e. inversions, for each for the same copy number variants. Each genome shows many megabases of variation within genomic regions not included in the hg19, underscoring the need for more de novo approaches to genome analysis.

PM16.28
Analysis of ciliome protein interaction networks to identify novel disease genes in a cohort of ciliopathy patients

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Ciliopathies represent a class of disorders caused by defects in the formation or function of the cilium. They result in a diverse yet overlapping range of phenotypes which are often chronically disabling and sometimes life threatening. Through previous large collaborative efforts, the proteins that comprise the cilia, the basal body and those that play a role in normal function have been identified and their interactions mapped, the so called ciliome. Although mutations in specific genes from within the ciliome have

been shown to give rise to specific ciliopathies, there are still a number of patients where no genetic mutations have been identified. We have sought to identify novel ciliopathy related genes in these patients through the analysis of exome data from a cohort of ciliopathy patients through the application of network analysis. We have used the known ciliome proteins and performed protein interaction analyses to explore novel, related networks of proteins whose cognate genes can be interrogated for causal mutations. This work has led to the identification of potentially novel findings.

PS16.29
Statistical procedure for pathway-guided GWAS analysis

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Genome-wide Association (GWA) studies have discovered thousands of polymorphisms affecting the risk of various complex traits. For most of these traits, exploration of the identified polymorphisms has revealed that the variants tend to be clustered near genes involved in few biological pathways. It therefore appears naïve to perform an unbiased GWA scan without taking pathway/network information into account. Post-hoc enrichment analysis only helps in interpretation of already discovered variants; it does not improve the power for novel discovery. It is now recognized that typical GWAS are under-powered to detect all associated variants due to a huge multiple-testing burden. This is particularly true for variants that are relatively less common and/or those with weaker effects that could have been missed in GWA studies conducted thus far. Pathway/network information, if properly harnessed, has the potential to improve the power of GWAS drastically. Here we propose a generic statistical procedure that can utilize pathway information a-priori while conducting a GWA scan. It automatically enhances the power to discover associated variants that are clustered in pathways. We demonstrate that, our procedure adapts to the data to maintain correct type-1 error (even if the pathway information is irrelevant). At the same time it can give huge power gains if the associated variants indeed cluster in pathways. Our method is fast and easy to implement even with summary-level data (p-values and odds ratios) from previous GWA studies. We also illustrate the performance of the procedure using published GWAS data from the Collaborative Association Study of Psoriasis.

PM16.30
Human Gene Family Resources at genenames.org

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The HUGO Gene Nomenclature Committee (HGNC) has assigned unique approved gene symbols and names to over 39,000 human loci to date. Approximately 19,000 of these are protein coding genes, but we also name pseudogenes and non-coding RNAs. Our website, genenames.org, is a searchable repository of HGNC approved nomenclature and associated resources. Approved gene symbols are based on names describing structure, function or homology, and where possible these are organised into gene groupings and families, many of which have specialist advisors who are experts in that particular area of biology. We are continually adding new gene families and currently have over 600 gene family pages. We have recently improved the display and content of these pages and sorted many of the families into hierarchies which allow genes to be browsed and downloaded at either a top level such as "G protein-coupled receptors", or at a more specific level, such as "Melanocortin receptors". The new gene family pages include family aliases, a family hierarchy map, a family text description, a graphical representation of protein domains for an example family member, and links to publications and relevant external resources. The gene families are fully searchable via the Search tool found at the top of each page at genenames.org and there is also a gene family index where users can browse through the full list of families. If you know of a gene family that you think we should include or update, please contact us via hgnc@genenames.org or talk to us during this meeting.

PS16.31
How far shall we go? Preliminary test on imputation of Italian isolated populations using an internal whole-genome reference panel

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The usefulness of isolated populations as models in large-scale GWAS studies is well known and their contribution helped unravelling the interaction between genetic variation and complex traits. The imputation approach based on reference panels led to new findings and to better insights in the range of low-frequency (<=5% MAF) variants thanks to the analysis of a complete scaffold of mixed populations haplotypes.
To exploit the peculiar characteristics of isolates and the advantages introduced by imputation, a subset of 987 individuals from three Italian Isolated Populations from INGI network was selected for Low Coverage Whole Genome Sequencing to draw an Italian specific reference panel.
The aim is to maximize imputation quality of Italian isolated cohorts and general populations (INCIPE , University of Verona) and provide a scaffold of low frequency variants haplotypes to complete the spectrum of South European human variation covered by the current standard reference panel.
Here is the first set of variants characterization based on a preliminary release of imputation panel collecting a batch of 568 samples: on average 36% of the total variations has frequencies < 1% whereas the comparison with outbred populations such as 1000G1 and UK10K2 highlighted an average of 15% of private variants. Accordingly, we expect to definitely power the genetic association studies on the large set of risk factors for common diseases.
1. 1000 Genomes Project Consortium, Abecasis, G. R., et al. (2012). An integrated map of genetic variation from 1, 092 human genomes. Nature 491, 56-65
2. www.uk10k.org

PM16.32
Application of RNA-Seq for gene fusion identification in leukaemia

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The development of Next Generation Sequencing (NGS) revolutionised different fields of science. RNA-Seq, a variety of NGS, is a method of transcriptome investigation, and a valuable tool in aberrant transcript identification. As such, it can be used to identify gene fusions in various types of cancers, regardless whether they are the underlying cause of cancer or the result of it. Gene fusions are associated with many types of leukaemia. We present gene fusion identification from RNA-Seq of samples taken from leukaemia patients, where more traditional methods of gene fusion identification, such as FISH, failed to identify correct translocations. We have found and confirmed with RT-PCR BCR-JAK2 fusion, molecularly similar to Philadelphia chromosome, in a patient with complex cariotype, and RAB20-ING1 fusion in another patient.

PS16.33
Linked data approach: some results and considerations from the first RD-Connect Bring Your Own Data meeting in Rome

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Patient registries, biobanks and bioinformatic data represent key resources for Rare Diseases (RD) to increase the chances of a timely and accurate diagnosis, improve patient management, tailor treatments and facilitate clinical trials. Unfortunately most RD registries and biobanks still keep their data "siloed" slowing down the process of discovery and development in RD research. Here we report some results and considerations of the Bring Your Own Data (BYOD) meeting, held in Rome in November 2014, in order to explore the potential of the "linked data approach" in RD research. The BYOD was co-organised by RD-Connect Linked Data (LD) and ontology task force, National Center for Rare Diseases Istituto Superiore di Sanità, Leiden University Medical Center, University of Aveiro, DTL/Elixir, BioMedBridges. The meeting brought together in particular LD experts and the members of RD-Connect, a six year project funded by the European Union but uniting researchers across the world. Participants were split into four groups and answered some cross-resource questions. Each group produced their first linked data to accomplish their objectives and managed to link data sets that were converted to resource description framework. LD technologies provi-

de a promising approach to connect registries and between registries, biobanks and bioinformatics data allows researchers to get more rapid responses to cross-resource questions improving the use of available information on RD. This prospect is slowed down by scepticism towards sharing data. We thank participants and contributors to the BYOD. BYOD was sponsored by RD-Connect and DTL/Elixir. CC, MR, SG equally contributed to the work.

PM16.34
Comparison of SOLiD sequencing data analysis pipelines

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Background. Software tools designed for the *Illumina* next generation sequencing (NGS) platforms are most documented. Alternatively, pipelines related to Life Technologies SOLiD platform, which use colour-space encoding, are not widely discussed in scientific literature. We applied several computational and variant calling pipelines for analysis of sequenced exomes of trios obtained by ABI SOLiD 5500 system. Using the same data sequenced by SOLiD we investigated proprietary *LifeScope's* pipeline and several other color-space competent mapping programs: *MAQ*, *SHRiMP* and *BFAST* coupled with *Genome Analysis Tool Kit* (GATK) for variant calling. *Annovar* program was used to annotate identified genomic variants.
Results. All methods covered 97% of the targeted regions. *LifeScope* and *SHRiMP* produced more mapped reads than *MAQ* and *BFAST*. To verify that called variants were not random we used transition/transversion (Ti/Tv) ratio. Ti/Tv ratio in all used pipelines was around 2.2 - 2.7. We compared how many known harmful variants from ClinVar and COSMIC databases were identified among called variants with at least x15 coverage by each investigated pipeline. *Lifescope* found the highest amount of known variants in these databases, while *MAQ* and *BFAST* pipelines called the least. Variants called by SHRiMP pipeline had lower mapping quality scores than other mapping programs.
Conclusion. Our comparison showed that *Lifescope's* pipeline is superior in analysing the SOLiD data. However, using several pipelines is encouraged because each pipeline creates a different view of the same variant data. This study is part of the LITGEN Project (VP1-3.1-MM-07-K-01-013) funded by the European Social Fund under the Global Grant Measure.

PM16.36
Clinical metabolomic profiling for the diagnosis of inborn errors of metabolism & undifferentiated genetic phenotypes

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Introduction: Clinical metabolomic profiling is a novel platform that allows for parallel testing of hundreds of metabolites in a single plasma specimen. We have validated this platform for common metabolic conditions compared with standard tests, including amino acid, organic acid, fatty acid oxidation, vitamin cofactor, pyrimidine biosynthesis, creatine biosynthesis, and urea cycle disorders.
Materials & Methods: A state-of-the-art mass spectrometry platform was utilized, and the resulting spectra were compared against a library of ~2,500 human metabolites. The analytes detected encompass numerous classes of important small molecule biomarkers, including fatty acids, amino acids, nucleotides, and other molecules sized 50-1500 Da. Validation samples and exome confirmatory samples were included.
Results: For the majority of IEM samples, classic pathognomonic analytes were among the most significantly elevated analytes detected; however, to achieve similar diagnostic outcomes with standard methods, many different biochemical tests are traditionally required. We found biochemical perturbations in pathways with no available clinical analyte testing such as citrate transporter deficiency. We were able to diagnose aromatic l-amino acid decarboxylase deficiency using plasma analytes (3-methoxytyrosine), which typically requires CSF neurotransmitter analysis. In a patient where exome revealed two VUS in the GABA transaminase deficiency gene (ABAT), metabolomic profiling was diagnostic revealing significantly elevated 2-pyrrolidinone, confirming the VUS as pathogenic.
Conclusion: Global metabolomic profiling is a novel test able to: (1) screen for common metabolic conditions in undifferentiated phenotypes, (2) reveal biochemical perturbations in pathways with no available clinical analyte testing, (3) diagnose disorders using plasma analytes that were heretofore only detectable in CSF, and (4) aid in interpretation of exome data.

PS16.37
MLPA 2.0 - Multiplex Ligation-dependent Probe Amplification on Illumina NGS platforms using a 600+ probe assay

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Introduction: MLPA, the multiplex PCR-based technique that has now become the standard in copy number variation, was developed by MRC-Holland in 2002. Although the method is used worldwide for the detection of copy number and methylation changes in human DNA, traditional MLPA has its limitations: an MLPA assay can contain a maximum of 60 probes and requires a minimum of 20-50 ng sample DNA of good quality.
We now introduce MLPA 2.0: a NGS-based MLPA variant enabling the use of 600 probes in a single reaction, with less stringent requirements regarding both sample DNA quantity and quality. This newly developed MLPA variant enables the creation of assays that screen a much larger proportion of the human genome. MLPA 2.0 assays can be used on all Illumina NGS platforms. As NGS-based sequence analysis still has its limitations when it comes to reliable detection of copy number variants, MLPA2.0 fulfills a yet unmet need. In this poster, we show the results of an MLPA 2.0 assay designed to complement sequence analysis for finding the cause of a hereditary predisposition to cancer. The assay contains probes for each exon of 26 genes involved in breast, colon, gastric and prostate cancer and melanomas, including BRCA1, BRCA2, MLH1 and MSH2.
Other MLPA 2.0 products that are currently in development include assays for newborn screening and the analysis of tumour-derived DNA.

PM16.38
The Human-Mouse: Disease Connection: a versatile tool for translational research discovery

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The mouse is a premier model system for investigating etiopathogenetic mechanisms and therapeutics for human disease. Mouse Genome Informatics (MGI, www.informatics.jax.org) offers a new translational tool, the Human-Mouse: Disease Connection (HMDC, www.diseasemodel.org), granting free, concurrent access to human-mouse genomic, phenotypic, and genetic disease information. Researchers can readily explore phenotypes and disease correlations, examine candidate genes, and evaluate experimentally defined mouse genotypes modeling a spectrum of clinically-relevant phenotypes. The HMDC portal empowers users to search from a human or mouse standpoint, using (1) genes or gene IDs for either species, (2) genome locations from either species, and (3) mouse phenotype or human disease (OMIM) terms. Alternatively, data can be uploaded from human or mouse VCF files. Users can input one or more genes, a region for QTL, multiple deletion regions, or enter phenotype/disease terms of interest (e.g., "CHARGE Syndrome", or "lymphopenia") using the optional autocomplete feature. Boolean searches are supported. Initial search results display in an interactive grid for visual comparison of phenotypes and diseases across multiple genes, phenotypes, and diseases. The grid employs color cues that reflect depth of human and mouse annotations, and grid cells are dynamically linked to underlying MGI data, including phenotypic detail, model-relevant publications, and global availability of mouse resources via IMSR (www.findmice.org). Alternate HMDC views include gene- and disease-centric information presented in tabular format. We present current content, optimized search and filter options, and integrated data mining approaches to effectively prioritize candidate genes and locate mouse models of preclinical value. Supported by NIH grant HG000330.

PS16.39
Discovery of novel markers for neural differentiation with Next-Generation Sequencing.

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Human neural stem cells (NSCs) are pluripotent cells that give rise to neurons, astrocytes and oligodendrocytes in the nervous system. They hold promise for treatment of brain and spinal injuries and diseases. However, very little is known about their regulatory mechanisms. Here we used Next-Generation Sequencing (NGS) to define the temporal transcriptome signatures of human NSCs. Cultured human embryonic stem cells (H9) were compared to induced NSCs at days 0, 7 and 14. Total RNAs were extracted and Ion AmpliSeq™ Transcriptome libraries were created for sequencing. The transcriptome profiles of H9 cells differed little between days 0, 7, and 14

while NSCs induced from H9 cells showed remarkable differences from day 0 to days 7 and 14 during differentiation. Hierarchical clustering also showed more robust sample classification for NSCs than H9 cells. Comparing the expression profiles of NSCs versus H9 cells, a total of 4001 and 4768 were differentially expressed at day 7 and day 14 respectively. We further clustered their expressions into 24 groups by Self-Organizing Map. A total of ~250 genes showed similar expression patterns to known NSC markers including NES, SOX1 and PAX6. These genes are enriched for neural differentiation related pathways and are potential candidates for novel NSC markers. Their expressions will be further validated by qPCR. In summary, we used NGS to construct a temporal transcriptome database of H9 cells and NSCs. We also developed an analysis pipeline to systematically identify potential novel NSC makers.
For Research Use Only. Not for use in diagnostic procedures.

PM16.40
Speeding up NGS analysis through local and remote computing resources

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Over recent years the explosion of NGS data and the need for ever faster computers to analyze them has pushed smaller laboratories to their limits. We previously presented a way for users of GensearchNGS to combine all their locally available computer resources to speed up the alignment process, by allowing multiple computers in the laboratory to work together. We now expanded this possibility for cases where the locally available computer resources are not sufficient. This has been done by including the possibility to use a remote cloud, such as the amazon cloud, to speed up the alignment process. If requested by the user, remote computing instances are dynamically created at the beginning of the alignment process and destroyed again once it ends, all transparently for the user. This gives the users the choice to align NGS data on his own computer, on all the computers in his local network or using the resources of a remote cloud. By providing the possibility to combine any of those 3 computing resources the user has the flexibility needed, particularly for smaller laboratories, to choose the optimal solution for their particular needs, be it in terms of required analysis speed or privacy data privacy policies. Possible solutions and approaches to the issue of privacy when sending data to the cloud are explored as well, such as the combination of raw sequencing data of multiple samples to obfuscate the data of the samples to be analysed.

PS16.41
Managing NGS Bioinformatics pipelines with bpipe and bpipe-config.

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Managing NGS Sequencing projects on large computation infrastructures can be challenging, especially if pipelines are managed with shell scripts: when a shell script fail, it is difficult to track where it failed and is even harder to restart the pipeline from the point of failure. Bpipe (Sadedin S et al., 2012), a platform for running big bioinformatics jobs that consist of a series of processing stages, tries to tackle these problems providing a framework to manage and launch bioinformatics pipelines in a controlled environment.
Herein we introduce bpipe-config, a software to generate pipelines ready for execution in bpipe, with a focus on Illumina sequencing environment.
The program bpipe-config make some check on the input files, generate a configuration file to launch bpipe on a cluster and create a pipeline using a generic template and the information stored in the SampleSheet file (i.e. reference genome, project name, sample name). When multiple samples are available, bpipe-config is able to generate a list of the input files that will be used for each sample.
As an example, we show our implementations for exome alignment, variant calling, variant annotation and WES project report. Each pipeline includes quality control steps (coverage, % unmapped reads, depth of coverage of variants) as well as a reporting infrastructure. The WES project report pipeline uses bpipe, R and the knitr R library to generate a complete report from a Whole Exome Sequencing project.
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PM16.42
CAVA: a variant annotation tool optimised for the clinical setting

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Next-generation sequencing (NGS) offers unprecedented opportunities to expand clinical genomics but also presents challenges, particularly in the annotation of indels, a highly important variant class in clinical genomics. Annotation in relation to a reference genome sequence, gene transcripts on different DNA strands and potential alternative variant representations has not been well addressed.
To address these challenges we developed CAVA (Clinical Annotation of VAriants) which is written in Python. CAVA is a fast, lightweight tool that can be easily incorporated into NGS pipelines. CAVA allows transcript specification, appropriately accommodates the strand of a gene transcript, which is crucial for consistent indel annotation, and flags variants with alternative annotations to facilitate clinical interpretation.
We evaluated CAVA in exome data and a clinical BRCA1/BRCA2 gene testing pipeline. CAVA annotated the ICR1000 exomes in 6.5 hours. Evaluation of 25,503 different indels revealed 79% had alternative representations in left-aligned and right-aligned data. Annotation of left-aligned data, as performed by many annotation tools would thus give clinically discrepant annotation for the 9,543 (37%) indels in genes transcribed from the forward DNA strand. By contrast, CAVA provided the correct clinical annotation for all indels. CAVA also flagged 9,177 indels with alternative representations of a different functional class, which may profoundly influence clinical interpretation. Finally, annotation of 50 BRCA1/BRCA2 gene mutations from a clinical testing pipeline gave 100% concordance with Sanger data; only 8/25 BRCA2 mutations were correctly clinically annotated by other tools.
CAVA is freely available from well.ox.ac.uk/cava. This work was funded by the Wellcome Trust Grant 098518/Z/12/Z.

PS16.43
Bioinformatic analysis of genes expressed in fetal cells circulating in maternal blood

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Fetal cells circulating in maternal blood are regarded as the main source of fetal cell-free DNA in maternal plasma which is examined in non-invasive prenatal diagnostics (NIPD). Understanding of biological processes associated with their release, viability and possibilities of their detection in maternal blood are therefore of interest for further development of NIPD.
In order to contribute to biological characterization of circulating fetal cells (CFC) we performed bioinformatic analysis of published gene set expressed in CFS and obtained the results documenting striking relationship between circulating fetal cells and endothelial cells. The results are in good agreement with the facts describing trophoblast deportation during pregnancy and its function as the fetal structure directly facing maternal blood.
Although there is the evidence of molecular similarity between circulating fetal cells and endothelial cells, the biological behavior of adult vascular endothelium practically excludes the presence of maternal endothelial cells in circulation. The situation is complicated by the presence of endothelial progenitor cells in circulation. Such incompletely described cells can undergo hematopoietic to endothelial transition and to regenerate vascular epithelium, therefore the spectrum of their molecular markers may overlap significantly with markers detected in blood cells, endothelial cells and circulating fetal cells.
It seems that a set of markers for effective CFC study in maternal blood for NIPD purposes could contain well known endothelial markers whose expression in CFC highly exceeds the expression in other blood cells.
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PM16.44
The Orphanet Rare Disease Ontology (ORDO): data modelling for rare diseases and genes

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As genetic knowledge becomes increasingly complex, there is an increasingly urgent need for reference tools that gather and integrate scattered phenotypic data in clinical databases and multiple medical terminologies in a normalised fashion, so as to improve their accessibility for health information systems and researchers.
Since 1997, Orphanet maintains a multilingual database of rare diseases (RD), based on the available
literature and on expert advice. Manually curated, Orphanet's data is comprised of a nosology (classification of RD), relationships (genes-diseases, epidemiological data, orphan drugs) and cross-references with other terminologies (MeSH, UMLS), databases (OMIM) or classifications (ICD10) in use. Genes are cross-referenced with other scientific databases (HGNC, OMIM, UniProtKB, Genatlas, Reactome, ensembl, IUPHAR). Orphanet's data is already freely available for download via the OrphaData platform (www.orphadata.org).
Data also needs to be made available to researchers in a machine-readable format, ready to be integrated with any technical environment. To address this need, Orphanet set up a collaboration with the European Bioinformatics Institute (EBI, Hinxton, UK) in order to produce the Orphanet Rare Diseases Ontology (ORDO), which provides a robust and consistent modeling of data and their semantic relationships, as well as interoperability standards with other scientific resources in use in both research and in public health. The Orphanet Rare Diseases Ontology is available on BioPortal (http://bioportal.bioontology.org/ontologies/ORDO) and Orphadata (www.orphadata.org).

PS16.45
Testing alternative methods to single-SNP GWAS analysis in family-based studies

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Genome-wide association studies (GWAS) have identified many single-nucleotide polymorphisms (SNPs) affecting complex traits. These SNPs often explain only a small amount of the total trait heritability, warranting the development of methods to find this ‚missing heritability'. The success of GWAS depends on strong linkage disequilibrium at the population level between individual SNPs and trait variants. Regional heritability mapping takes into account the associations of causative variants with all SNPs in a region, allowing the identification of regions affecting the trait where individual SNP effects are small and would be missed by single-SNP GWAS. Linkage analyses utilise associations between SNPs and trait variants within families and not at the population level. Linkage analyses have been successful in identifying regions associated with monogenic traits, but less so when analysing polygenic traits in the GWAS age.
Here, we performed in parallel variance components linkage analysis, regional heritability analysis and GWAS on about 50 quantitative traits of public health importance (e.g. blood biochemical traits, anthropometric traits) in several isolated and cosmopolitan family-based populations.
We identified promising linkage peaks (LOD scores of 4-6) for several traits, in individual populations, with little overlap with known single SNP GWAS regions. Regional heritability frequently flagged regions picked up by GWAS, but also identified novel regions that lie within, or near, genes that are good candidates (based on function) for affecting a trait.
Replication and meta-analysis results across studies are being examined and the most promising regions will be followed-up using haplotype analyses and exome- and whole-genome sequencing data.
Funded by MRC PhD studentship.

PM16.46
CliMGuiDeS: online platform to develop clinical management guidelines for rare conditions

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Rare diseases, defined as occurring at a frequency lower than 5 per 10,000 people, will affect 1 in 17 people at some point in their life. Thus, although individually are rare, collectively are very important. There are around 6000 rare disease, many are life threatening and/or require continuous care.
Their optimum management, achievable with specialised clinical management guidelines, improves quality of life, and has financial benefits for the individual, their family and society. However, because of their rarity, the evidence base for some of their therapies and treatments is poor. Thus, guidelines need to be carefully drawn up, based on available evidence and expert consensus opinion and ensure that those with intimate knowledge of the natural history of the disorders are engaged in the process.
Unfortunately, its development is a costly process, requiring expert knowledge and time, as the Dyscerne project demonstrated previously.
CliMGuiDeS aims to create an all-in-one solution to streamline the development of management clinical guidelines for rare conditions, through the establishment of an online platform providing user-friendly environment with detailed information and tools to facilitate the completion of each step during this complex process. This platform will be globally accessible, and free to establish and sustain; it will not require specific (bio)informatics knowledge, operative-system, server, data-storage, or payable software. Further, to prove that effective work and innovative approaches can provide solutions even under the most challenging situations, this platform will be developed within just three months by one student.

PS16.47
Cafe Variome: Comprehensive Data Discovery for Rare Disease Diagnostic Networks and Research Consortia

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Vast amounts of useful mutation and phenotype data are not being openly shared - often for good legal, ethical or competitive reasons. The full value of these data is therefore never realised, and this fundamental ‚data sharing' roadblock cannot be solved by any technical wizardry that emphasises sharing.
Instead, the Cafe Variome approach changes the nature of the problem to one of enabling data discovery - i.e., making the ‚existence' rather than the ‚substance' of the data accessible. All records in the system can be made discoverable to help users comprehensively establish what useful content exists. The software facilitates the subsequent supply of these discovery 'hits' depending on who the data requestor and the data owner are.
The main use case being supported involves networks of rare disease diagnostic laboratories or research consortia within which members share an interest in certain causative genes/diseases, know/trust each other to different degrees, and wish to have a full picture of what records exists across the collaborating network. The first of these federations (for cardiovascular disease) has recently gone live and a number of other groups are currently trialling the software.
The development is lead by the University of Leicester, UK. The team and the concept are contributing to a number of major international initiatives focussed on maximising data use. To be compatible with these and other future initiatives all aspects of the Cafe Variome software are designed to be maximally flexible, enabling any data fields, ontologies, federation architecture, discovery search options, and data complexity to be handled.

PM16.48
Creation of ‚IRDiRC Recommended', a label to be used to highlight tools, standards and guidelines contributing to IRDiRC objectives

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Following intense brainstorming exercises conducted by the International Rare Disease Research Consortium (IRDiRC), the Executive Committee decided to establish a label to highlight tools, standards and guidelines contributing to IRDiRC's objectives, namely to deliver 200 new therapies to the market by 2020 and the tools to diagnose most rare diseases.
The 'IRDiRC Recommended' label is a quality indicator, based on specific criteria. Any tool/standard/guideline compliant with the required criteria is entitled to receive the label. 'IRDiRC Recommended' is a public label which could, and should, be made visible on and by the resource, giving users an assured guarantee of its quality/appropriateness. The selected tool/standard/guideline must be fundamental to the rare diseases research and development community.
To receive the label, the tool/standard/guideline must fall within IRDiRC's focus, mission and requirements. It should have a development and maintenance team, and be functional and accessible with minimal downtime. It must be well-documented with clear terms-of-use and licence policies, and should adhere to all relevant ethical and privacy policies. The tool/standard/guideline should have implemented quality control and life cycle management processes, be financially viable for three years following its label accreditation and provide documented evidence of its core impacts (e.g. number of users, number of visits). Moreover, the tool/standard/guideline

should be freely available and must not be a commercial product.
Applications for the label will be peer-reviewed and submitted for approval by the IRDiRC's Executive Committee. Applications will be received and reviewed on an ongoing basis.
FP7/2007-2013, grant agreement n° 305207 "Support for international rare disease research to serve the IRDiRC objectives (SUPPORT-IRDiRC)".

PS16.49
Genomic, transcriptomic and miRNomic integrative analysis of recombinant inbred rat models of metabolic syndrome

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We have previously established a genetically designed set of recombinant inbred rat strains PXO as a model of metabolic syndrome. Aim of this study was to compare, on genomic, transcriptomic and miRNomic levels, two PXO strains showing highest contrast in metabolic syndrome parameters.
At genomic level we have compared > 20,000 SNPs between PXO3-1 and PXO3-2. Both RNA and miRNA were isolated from liver, visceral adipose tissue and muscle (m. soleus) of adult males of both strains and its integrity was checked by Agilent 2000 BioAnalyzer. The transcriptomic and miRNomic assays were run using Affymetrix® Rat Gene 2.1 ST Array Strip and Affymetrix® miRNA 4.1 Array Strip, validated by qPCR. Resulting data were subjected to systems biology-level analyses using Partek Genomics Suite and Ingenuity Pathways Analysis.
PXO3-2 showed impaired glucose tolerance, higher TG and HDL-cholesterol together with lower adiposity compared to PXO3-1. On the genomic level, we have identified polymorphic regions on chromosomes 1, 3, 5, 8, 12, 16 and 19 totalling at 3.2% of genome. After correction for multiple comparisons, there were 1133, 236 and 29 differentially expressed transcripts between PXO3-1 and PXO3-2 in liver, visceral adipose tissue and muscle, respectively. Integrative pathway analysis revealed networks likely to underlie the observed metabolic differences including Ppara, Ppard, Insig2, Por a Srebf2 genes as their major nodes.
Using integrative approach of genomic, transcriptomic and miRNomic analysis in, we have identified major biological networks contributing to the pathophysiology of several aspects of metabolic syndrome in the recombinant inbred model set.

PM16.50
Identification of rare variants by exome analysis in multiplex families with rheumatoid arthritis.

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Introduction: Some common variants potentially involved in rheumatoid arthritis (RA) have been identified by genome wide association analysis. However, the genetic component of RA is not fully defined, and the identification of rare variants could help us to characterize a part of the missing heritability.
Materials and Methods: To identify rare RA-associated variants, we have analysed exome sequences from 30 individuals belonging to multiplex families in order to enrich the genetic component: 16 individuals (11 affected and 5 unaffected) from five families with at least four cases of RA (RA_fam); and 14 individuals (11 affected and 3 unaffected) from four families with at least four cases either of RA or other autoimmune diseases (RA_AID_fam).
Results: Exonic DNAs were captured with Agilent SureSelect Human All Exon V5 kit and sequenced with an Illumina HiSeq2000 sequencer. After alignment, quality filtering, targeting of captured regions and genome annotations to select variants according to their minor allele frequency (less than 1% or absent in databases) and their in silico predicted risk, we identified some rare variants present in all RA cases in one RA_fam but absent in unaffected relatives and in other individuals from both types of families. Familial and inter-familial analysis of these variants is currently ongoing.
Conclusions: Selected variants will be further validated by genotyping extended to all available members of each family in order to perform segregation analysis.
This work was supported by Fondation ARTHRITIS.

PS16.51
Large Scale Metabolomics identifies Dysregulated Metabolic Pathways in Rheumatoid Arthritis and Its Sub-types

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Background
Rheumatoid Arthritis (RA) is a systemic chornic inflammatory disease of unknown etiology predominantly affecting the synovial joints. Although antibodies are now helpful in making early diagnosis of RA, novel diagnostic and prognostic markers are still needed to improve both diagnosis and treatment. Previous studies indicated the importance of metabolic profiling in uncovering details of pathomechanisms of RA. Untargeted metabolomics approach, together with metabolic networks, allows a deeper investigation of pathways associated with RA.
Objectives
Using an untargeted metabolomics approach, we investigate metabolic dysregulation in RA cases versus controls and in the sero positive and sero negative sub-types versus controls. Metabolic networks are further constructed using correlations between metabolites, to explore metabolic mechanisms underlying RA.
Materials and Methods
Blood and serum samples from 236 subjects of Arab ancestry were collected. Metabolic profiles were identified using the untargeted approach by Metabolon Inc.. Regression models, that correct for covariates were used for identifying metabolites associated with RA, as well as those differentiating sero positive from sero negative cases. Metabolic networks are then constructed and overlaid on identified metabolites.
Results
Several steroids showed significant decrease in RA patients, among which DHEAS has been previously reported. Other steroids which were not reported before in relation to RA were also identified and were found in the DHEAS metabolic subnetwork. Sero positive and sero negative RA cases further stratified the RA associated metabolites into two subsets.
Funding
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PM16.52
RNA-Seq data analysis pipeline

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Next-generation sequencing has become the main technique for investigating the transcriptome. Although microarrays have been successfully used over years they have several limitations and the decreasing costs of NGS have led to a shift from microarrays to RNA sequencing (RNA-Seq).
We developed a pipeline to analyze RNA-Seq data and a database to store important metrics. The pipeline is a combination of Perl scripts and public available software packages (i.e. GEMmapper, HTSeq, DESeq2). It was integrated in an already existing exome sequencing data anlysis pipeline and its flexible and modular structure allows for an easy integration of new features which is important to cope with emerging issues in the constantly enhancing field of Next-generation sequencing.
As a standard feature, the pipeline performs split read alignment for each RNA-Seq sample and counts the number of reads within annotated features. To enable the detection of unwanted bias we calculate numerous quality metrics like amount of sequence, mapping rate, duplication rate, inter- vs. intragenic rate or rRNA rate. Subsequently, depending on the experimental design and possibly detected biases one can adapt parameters for downstream analysis such as differential gene expression or pathway enrichment analysis.
Overall, we applied the analysis pipeline to more than 700 RNA-Seq samples. On average, over 6 Gb of sequence was generated per sample resulting in more than 60M reads. We yield an overall mapping rate of about 99% and approximately 82% of the reads map within annotated exons. The rRNA rates are consistently below 1%. Furthermore, duplication rates vary considerably between standard (~20%) and low-input (~35%) RNA-Seq protocols.

PS16.53
FEATURES OF THE FIRST REFERENCE SEQUENCE OF SAUDI

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A total of 2311051 unique variants were detected in the reference subjects that use for establishment of Saudi reference
sequence. however; the Alignment of the genomics data of the targeted subject was performed against human genome (hg19) by
hybrid approach. it was noticed that more than 10000 unique indels and almost 16000 structural variants were detected the
reference subjects
Our previous data that showed more than 14% of the SNPs were unique among 32 subjects was also present at our proposed
reference . A de novo assembly of 9,894 contigs sequences was not represented in NCBI reference genome.
Conclusion: De novo assembled and analyzed full Saudi Arabian individual genome of showed more than 231K
polymorphisms that is sole compare to the reference sequence.

PM16.54
A Micro-fabricated Single Cell Isolation System To Process 1000s Of Individual Cells For Genomics Applications

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Understanding biological heterogeneity at a single-cell level requires new technologies that miniaturize and automate the isolation of thousands of individual cells and process these individual cells for downstream applications such as NGS or qPCR. Microfluidic technologies have streamlined single cell genomics applications but can do at best 96 individual cells at a time and costs over $20/cell. Droplet-based technologies have been shown to process thousands of cells but also hold several productizing challenges including handling emulsion oils and optimizing reaction efficiencies at picoliter volumes. In its first product version, WaferGen aims to increase throughput ~50 fold while simultaneously decrease cost at least 10-fold by engineering a simple device based on its proprietary, scalable, micro-fabricated chip, SmartChip, which contains an array of 5184 individual polymer-coated nanoliter wells drilled into a metal alloy substrate. The SmartChip platform has been used successfully for qPCR, genotyping and targeted sequencing applications and is proven for its flexibility in processing multiple samples in a single chip using the MultiSample NanoDispenser (MSND), a microsolenoid controlled reagent dispenser that can dispense volumes as low as 40 nl into the SmartChip wells. We will present data to support the reproducible dispensing of individual cells at a >80% occupancy rate (~4000 cells) and barcoding individual cells with unique molecular identifiers by adapting SCRB-seq, a Single Cell RNA Barcoding and Sequencing strategy to estimate 3' differential gene expression (http://dx.doi.org/10.1101/003236).

PS16.55
The functional annotation of genetic sequence variants using FATHMM-MKL

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We propose a novel approach for predicting whether single nucleotide variants (SNVs) are functional in human disease. We use an integrative classifier that weights component data-types according to their relative informativeness, and assigns a confidence measure to each predicted class label. We evaluated our approach using pathogenic germ-line SNVs from the Human Gene Mutation Database, and neutral SNVs from the 1,000 Genomes Project. The majority of disease-associated sequence variants lie in non-coding regions, but currently only two other recently proposed predictors exist for these variants. Using a weighted combination of four data sources, our method significantly outperforms both competing methods, and is currently state-of-the-art. The most highly weighted data source is sequence conservation across species: a variant in a highly conserved region has a higher probability of disease association than variants in regions of high variability. If we focus on examples with a confidence measure above 90%, the classifier achieves 96% test accuracy while making predictions on nearly 40% of examples; at a 95% confidence cutoff, accuracy increases to 98% with predictions for nearly 16% of examples. In coding regions, our model uses a weighted combination of 10 sources of data and is as accurate as the top competing algorithms. We have also devised disease-specific predictors and provide a web-browser for visualising the results from these studies.
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PM16.56
Improved Detection of Low Level Sequence Variants by Sanger Sequencing using New Variant Caller Software

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Sanger sequencing using capillary electrophoresis (CE) has been considered gold standard for identifying disease-causing mutations due to its robustness, low error rate, ease of use, visual display of the electropherogram, and low cost per sample and target. Homozygous and heterozygous germline mutations are reliably detected using DNA sequencing analysis software such as the Applied Biosystems Variant Reporter™ software. However, somatic variants with an allelic proportion of 25 % or less are often not „called" by the software and thus escape awareness if not detected by careful visual inspection of the electropherogram. With the rapid adoption of next generation sequencing technology (NGS) and its use for characterization of specific and discrete mutations in tumor samples, a need has emerged to establish an orthogonal technology for reliable and sensitive detection of somatic mutations which may occur at proportions of 10% or lower compared to the normal allele. To this end, we have developed innovative algorithms, software, and a protocol that specialize in the detection of minor mutations by Sanger sequencing. Using panels of prepared mixtures of minor alleles in the range of 2.5%, 5%, 10% and 20 %, we have achieved 92% sensitivity and 98.5 % specificity for detection of mutations present at the 5 % level with high quality data. In conclusion, we have demonstrated that standard protocols for fluorescent dye terminator Sanger sequencing in conjunction with the new Variant Caller may enable identification of de novo somatic mutations to a level of 5% which will also be useful for the confirmation of minor variants identified by NGS platforms.

PS16.57
The protein-protein interaction network of the human spliceosome

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Splicing decisions are controlled by two major determinants, the pre-mRNA sequence and its inherent signals as well as the protein complement of the spliceosome. A major difficulty concerning the spliceosomal functional analysis arises from the large number of proteins involved and their dynamic interactions within spliceosomal sub-complexes. The aim of this study is the in silico reconstruction of the protein-protein interaction (PPI) network of the human spliceosome.
We identified the proteins that have been isolated as subunits of the human spliceosome and/or its subcomplexes, based on all the relevant publications. Direct PPIs between spliceosomal components were retrieved from the human interactome knowledge base, PICKLE_DB augmented with PPIs for the D. melanogaster and C. elegans orthologous to human spliceosomal proteins from the DroID and Worm Interactome Database. A thorough manual curation of the acquired data and the supporting publications ensured a final set of confidently direct PPIs.
The proteome of the human spliceosome comprises 630 proteins, 60% of which can be assigned to specific spliceosomal sub-complexes. The reconstructed spliceosomal PPI network, which follows the power law distribution, consists of 457 nodes and approximately 1600 edges. Approximately 25% of the proteins-nodes have been associated with genetic diseases, making the reconstructed network a valuable platform for suggesting documented functional relationships between genes, diseases and the topological structure of the interactome.
Acknowledgments:
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PM16.58

Hadoop-based NGS Analyzer for Predicting Genomic Structure Variations

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It is almost impossible to store or analyze such large scale NGS data with a traditional method on a commodity server. Hadoop is an alternative to this requirement. We aim to address the issues involved in the large scale data analysis on the cloud in bioinformatics. Accordingly, we propose a Hadoop-based algorithm HAVS for predicting genomic variations at least 1000 bp and related to diseases.
Our algorithm consists of 3 steps in MapReduce workflow. The first step is to classify the sequences as normal sequences, of which the direction of paired-end reads is correct and in which the mapping distance belongs to the range of ±2.7SD standard deviation of the mean fragment size of the reads, and the sequences that do not belong to the range as variation. The second analysis process is to compute the mean insert size of sequences by structural variation types for only the sequences regarded as structural variation. The third analysis step is to cluster the structural variation sequences on the basis of the information computed in the above analysis process, and to predict a breakpoint in which structural variation occurred.The algorithm covers the structural variation types such as segmental duplication, copy number variation, translocation, inversion, insertion, and deletion. The experiments are performed on Hadoop clusters with the proposed algorithm and the YRI sequencing data provided by the 1000 Genome Project. The experimental results show that our proposed algorithm based on Hadoop is more accurate, efficient, and applicable to the prediction of genome structural variations.

PS16.59

Resolving Complex Structural Genomic Rearrangements using a Randomized Approach

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Structural variants (SVs), defined as the rearrangement or removal of genomic regions, are major sources of genetic diversity in human populations and directly responsible for the pathogenesis of numerous diseases. Many studies have been conducted in the past decade to discover and analyze SVs, however these have predominantly focused on unbalanced (copy number variant) events involving only one or two breakpoints. In contrast, more complex rearrangements resulting from multi-step or overlapping events involving three or more breakpoints have received considerably less attention or have been incorrectly interpreted. We have developed an algorithm, SVelter, which accurately identifies and resolves both simple and complex rearrangements using whole genome, paired-end sequencing data. This method first detects regions of the genome that are suspected to involve a structural variant by clustering sets of candidate breakpoints using aberrant read pair and split read mappings. The resulting segments are then iteratively rearranged in a randomized fashion and scored against null models of expectation based on library insert length, read orientation, sequence depth, and spanning coverage. We applied our algorithm to simulated genomes containing both simple and complex events and show a comparable accuracy for simple deletions, duplications and inversions, as compared to modern SV calling methods. However, SVelter outperforms these approaches on more complex rearrangements, showing a higher sensitivity without significant loss of specificity across different levels of sequence coverage as assessed using individual haplotype structures. We have also reexamined well-characterized genomes from the 1000 Genome Project (1KGP) that have been deeply sequenced and compared our results to the reported structural variants in these samples.

PM16.60

Sexpression analysis of >1,700 Finnish individuals reveals sex-dependent transcriptional differences in whole blood for immune system processes, response to stress and lipid metabolism

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Background: Sex-differences in gene transcription levels have been studied in specific diseases and specific tissues. It is important to identify normal sex-dependent differences in transcription levels, which can only be studied in population cohorts of large enough size.
Methods: We studied differences in autosomal gene expression levels between males and females in two Finnish population cohorts, the Young Finns Study (YFS, N=1255) and DILGOM (N=518), having genome-wide transcriptional coverage (>30,000 transcripts) from whole blood detected with Illumina HumanHT-12 BeadChips. The cohorts were analyzed separately using linear regression and meta-analyzed with random-effects meta-analysis. Covariates included age, BMI and smoking, and women using contraceptive pills or pregnant were excluded from the analyses.
Results: After correction for multiple testing, 992 autosomal transcripts (3.5%) in 949 genes showed differential expression between sexes. Gene enrichment analysis revealed significant enrichment of six Gene Ontology (GO) Terms including immune system process (p-value=4.9e-25) and response to stress(p-value=9.8e-6). Altogether 77 genes with GO terms related to lipids or lipoproteins showed differential expression in males and females. Interestingly, the Lipase A (LIPA) gene showed higher expression levels in males (0.54 increments in SD), with p-value 5.34e-28. This gene encodes lysosomal acid lipase, an essential enzyme for the hydrolysis of triglycerides and cholesteryl esters in lysosomes.
Conclusions: A high number of genes show different levels of expression between sexes when studied in a population cohort setting. These analyses can potentially pinpoint complex disease risk factor genes (e.g. LIPA) that may have a differential effect to the risk factor between males and females.

PS16.61

Variant data from more than 100,000 samples at the European Variation Archive

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The European Variation Archive (EVA; www.ebi.ac.uk/eva) at EMBL-EBI is a one-stop-shop for variant data from all species. EVA contains more than 300 million variants from over 100,000 samples and our resource is growing at an increasing rate month on month due to submission of data from researchers interested in our service and the in-house addition of high-value datasets.
EVA data is annotated using a variety of methods, including VEP, for both the complete and basic GENCODE genesets. Data statistics are calculated both intra and inter study, and we also permit views of submitter provided annotations. We shall present the ways in which users can mine these data using filters on the website to construct both study-centric and global queries, filtering on any combination of species, methodology, variant type, phenotype, consequence or allele frequency and how results from these queries can be downloaded in a variety of formats. Additionally, EVA provides a comprehensive RESTful web-service, to allow programmatic access, and hence the integration of these data with other resources such as Ensembl Variation and Uniprot, and this shall also be presented.
Data submitted to EVA is brokered to NCBI (dbSNP or dbVar) as appropriate. Additionally, EVA has imported all dbSNP human variants and is continuing to load other species. Finally we shall present how EVA, supported by our close integration with ClinVar, accepts submission of clinically relevant data and describe the tools we offer that allow queries to be constructed against the complete clinical dataset of more than 100,000 variants.
Please send all questions, comments and/or submissions to eva-helpdesk@ebi.ac.uk

PM16.62

Human Variation Database in Japanese Integrated Database Project

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We have established the Human Variation Database (http://gwas.biosciencedbc.jp/cgi-bin/hvdb/hv_top.cgi) in order to achieve continuous and intensive management of Japanese GWAS data, CNV data and variations identified by NGS and other experimental methods. The scope of the registry includes not only single gene disease causing variations and multifactorial disease associated variations, but also variations associated with drug responses and infectious susceptibilities. Since some phenotype-related variations are recognized to be different among populations, the reference genomes have been built for different populations. Variations include short/long insertions and deletion and structural variations as well as SNP/SNV.

Since vast amount of knowledge about variation-phenotype relationships are varied in the scientific literature, these data have been extracted by manual curation to organize the phenotype-related variations and improve our understanding of disease mechanisms and disease-disease relationships. More than 30,000 disease-related variation entries are currently registered. Since many characteristics of the HLA regions are different from other genomic regions, HLA-DB has also been constructed. Although only variations in germ-line cells are targeted in the database, it also provides links to driver mutations archived in the widely used databases such as COSMIC in order to clarify the relationships between the driver mutations in various cancers and germ-line mutations. Furthermore, pathway data are also provided to facilitate the understanding of epistasis and relationships between related diseases.

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PS16.63
Likelihood-based inference of relationship for family exome data

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Whole exome sequencing (WES) analysis has become a powerful strategy to search for causal variants underlying rare Mendelian disorders. However, the identification of the disease-causing mutation among thousands of potentially functional sites can be challenging and often starts with linkage analysis to identify the chromosomal location of the potential disease gene. This strategy depends completely on correct pedigree information and may lead to false discoveries in a case of sample swaps or label mix-ups.
However, detailed knowledge about relatedness is not only a prerequisite for linkage analysis but is also becoming more and more important for genome wide association studies. While a sample mix-up is severely corrupting any linkage analysis, the inclusion of loosely related individuals in a case-control group scenario may result in spurious associations.
We created a strategy that combines two approaches to detect population substructure and predict complex pedigrees from genomic data. On the one hand we developed an algorithm based on likelihood ratios to discriminate different orders of relatedness between sample pairs of WES family data (e.g. siblings, parent-child, unrelated). On the other hand we applied a similarity metric on a collection of individuals to characterize their genetic population substructure on a group level by comparing each sample to genotype data provided by the 1000 genomes project.
By this means we are able to predict the correct pedigrees from exome samples of multiple generations as well as to detect cryptic relatedness in a cohort of an exome study.

PS17.01
Hair e-QTLs - delineating the genetic basis of gene-expression in human hair follicle

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Human scalp hair plays an important role in our social and cultural life and its undesirable loss is often perceived as psychologically stressful. This has provoked many studies on the identification of genetic risk factors that predispose to hair loss disorders such as alopecia areata and male-pattern baldness. However, the biological context in which these genetic risk factors exert their biological effect is often unknown. Here, the analysis of genetic variants that influence gene expression (eQTLs) has gained major importance. The aim of our present study was to systematically map eQTLs in human hair-follicle that can be used to functionally annotate genetic risk factors for hair-related traits. Genome-wide genotyping of blood-DNA and transcriptome profiling of hair-follicle-RNA was performed on Illumina's OmniExpress- and HT12v4-arrays. After imputation of genotypes and quality control, a total of 6,593,881 SNPs and 14,687 expression-probes from 97 individuals remained for analysis. The genome-wide eQTL-analysis identified 2,883 independent cis- and 224 trans-eQTLs. A comparison with published eQTL-data from peripheral blood and brain revealed hair-follicle-specificity for about ~40% of the cis-eQTLs (N=1,159). The strongest hair-specific effects were observed for SNHG8, MRPL43 and XYLT1. IPA-pathway-based analysis revealed an enrichment for hair-eQTL-genes in several pathways. Among the top findings are the androgen- and prolactin-signaling-pathway which have been implicated in hair growth (de)regulation and the melanocyte development and pigmentation signaling. It is hoped that integration of the present data with GWAS-findings will help to pinpoint novel candidate genes and pathways and to further elucidate the biological mechanisms that contribute to the development of hair loss disorders and hair-related phenotypes.

PM17.02
C9orf72 epigenetic modifications in Italian amyotrophic lateral sclerosis patients

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The hexanucleotide repeat expansions (REs) in the non-coding region of *C9orf72* are by far the most frequent cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia, accounting for a large proportion of both familial and sporadic cases. The basis for *C9orf72* carriers clinical heterogeneity remains unknown. Recently the association between *C9orf72* promoter methylation and histone trimethylation with its transcriptional silencing and with decreased accumulation of RNA foci and dipeptide repeat protein aggregates suggested that epigenetic modification of *C9orf72* could mitigate disease pathogenesis.
To consolidate this hypothesis we studied *C9orf72* promoter methylation in 44 *C9orf72*-positive and 22 *C9orf72*-negative ALS patients using bisulfite conversion coupled with next generation sequencing (NGS). When possible, we also performed quantitative PCR on RNA isolated from blood to determine *C9orf72* expression.
As a result from BS-sequencing, 91% of *C9orf72*-negative patients were unmethylated, while 32% of carriers had more than three methylated CpG sites. Performing NGS, we estimated with accuracy the percentage of methylation at each CpG site; this allowed us to refine the previous classification, dividing the samples in five different classes and concluding that only 27% of carriers had medium or high degree of methylation. While methylation levels didn't correlate with age at onset, they were mild correlated with disease duration and were inversely associated with repeats length. Quantitative PCR confirmed the correlation between *C9orf72* promoter methylation and lower levels of *C9orf72* RNA. Hence our data support the hypothesis that epigenetic silencing of mutant *C9orf72* and its subsequent diminished expression are associated with less aggressive disease.

PS17.03
Monozygotic female twins discordant for Beckwith-Wiedemann syndrome

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We present a case of monozygotic, monochorionic female twins discordant for Beckwith-Wiedemann syndrome (BWS) with detailed clinical description and DNA methylation analyses in order to provide further clinical and molecular data to the understanding of the mechanisms leading to this phenomenon.
The twins were born at gestational week 36+6 after a planned caesarean sectio due to an estimated weight difference of 1 kg between them.
Twin A was clinically unaffected with a birth weight of 2,739 g, while twin B had clinically BWS with a birth weight of 3,542 g, an enlarged tongue, nevus flammeus, bilateral ear pits, an umbilical hernia and neonatal hypoglycaemia
DNA extracted from peripheral blood, and buccal swabs of both twins, were analysed at a number of differentially methylated regions (DMRs) including DMR2 (KvDMR1) of the BWS region at chromosome 11p15.5. Partial loss of methylation (LOM) of the maternal allele of DMR2 in DNA extracted from blood from both the clinically affected as well as the phenotypic normal twin was identified. On the other hand, partial LOM of DMR2 in DNA from buccal swab was only identified in the clinically affected twin but not in the clinically unaffected twin.
Clinical follow-up at 1½ year of age and results of the extensive molecular analyses including the methylation profile of other imprinted loci will be presented.

PM17.04

Detection of 11p15 imprinting defects in patients with Beckwith-Wiedemann and Russell-Silver syndrome by Methylation-Specific Multiplex Ligation-dependent Probe Amplification method

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Beckwith-Wiedemann syndrome (BWS) and Russell-Silver syndrome (RSS) are clinically heterogeneous disorders associated with overgrowth and growth retardation respectively. These conditions are caused by genetic or epigenetic changes that modify expression of genes in the imprinted region of chromosome 11p15.5. Approximately 60-70% of the patients have imprinting abnormalities at one of two imprinted domains H19DMR (imprinting center 1, IC1) or KvDMR (imprinting center 2, IC2). Loss of methylation at IC2 on the maternal chromosome is found in 50% of persons with BWS. Hypomethylation at IC1 on the paternal chromosome is identified in 35%-50% of individuals with RSS. Other causes of BWS and RSS are uniparental disomy (UPD) of chromosome 11p15.5 (BWS) and 7 (RSS), deletion/duplication, inversion and translocation of 11p15.5 and mutations in the CDKN1C gene.

Methylation-Specific Multiplex Ligation-dependent Probe Amplification (MS-MLPA) method enables detection of aberrant methylation of the KvDMR and H19DMR domains in the 11p15 BWS/RSS region. Also, it can be used to detect deletions/duplications in the same region.

We have analyzed six patients with BWS and four patients with RSS by MS-MLPA method. MS-MLPA analysis has shown hypomethylation pattern in gene KCNQ1OT1 in all subjects with BWS. In subject with RSS, MS MLPA revealed hypomethylation pattern in gene H19.

MS-MLPA provides robust and simple detection of epigenetic and genomic alterations at 11p15.5 as it detects copy number changes and DNA methylation alterations including those resulting from UPD. Therefore, MS-MLPA analysis should be offered in all cases of BWS/RSS as a first line test.

PS17.05

Integrating genome-wide methylation and genetic data for understanding biomarker regulation

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An important aspect of modern medicine is the use of biomarkers to estimate disease risk. A better understanding of the importance of genetic and epigenetic factors in the regulation of biomarker expression, is crucial for discerning the role of biomarkers in diseases as well as the causes of interperson variation of biomarker levels.

In this study, genetic and epigenetic data are integrated to study factors that influence circulating levels of 144, well-established and exploratory, protein biomarkers for cancer and cardiovascular diseases. An epigenome-wide association study (EWAS) was performed using DNA methylation data from >470K CpG sites in 729 individuals and a genome-wide association study (GWAS) was performed using >8 million genotyped and imputed SNPs in 1033 individuals from a population-based cohort.

Results revealed EWAS significant CpG sites and GWAS significant SNPs (Bonferroni adjusted p-values <0.05) for 45 and 36 biomarkers respectively. In total 189 CpG sites, distributed over 130 loci and 4361 SNPs at 31 loci were identified. Many SNPs are located within or close to (<1Mb) the gene encoding the respective biomarker. A subset of the EWAS and GWAS signals overlap, suggesting that these represent the same underlying regulatory factor, while other signals characterize different regulatory factors. Some CpG sites are associated with multiple biomarkers, e.g. one in the inflammatory gene NLRC5.

Integrating GWAS and EWAS data gives a unique possibility to study their respective roles in regulating protein biomarkers. This knowledge is important in order to better understand the role of biomarkers in disease development and progression.

PM17.06

Potential of CDH1 promoter hypermethylation as a biomarker in early detection of cervical cancer

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E-cadherin is a transmembrane glycoprotein encoded by CDH1 gene and plays a pivotal role in cell-cell adhesion, suppression of tumour invasion, metastasis and participates in cell signaling. Intensive studies of many types of human cancer found reduced or lost expression of E-cadherin that might be also linked to the epithelial-mesenchymal transition (EMT) whereby epithelial cells can convert to mesenchymal cells via a multiple-step process. Our hypothesis was based on the fact whether the methylation of CDH1 gene is detectable in cervical swabs and has potential in cervical cancer screening. We studied CDH1 promoter hypermethylation in a spectrum of cervical specimens including normal cervix (n=47), low-grade (LSIL, n=34) and high-grade squamous intraepithelial lesions (HSIL, n=46) and invasive cervical cancer (n=13). The presence of CDH1 promoter hypermethylation was investigated by methylation-specific PCR (MSP-PCR) and its effect on CDH1 gene transcription was evaluated by relative quantification. We found out the presence of CDH1 hypermethylation in 20.6% (7/34) of LSIL, 21.7% (10/46) of HSIL and 46.2% (6/13) of cancers. We confirmed that the presence of CDH1 promoter hypermethylation was significantly associated with formation of cervical lesion resp. cancer (p=0.0005) and its expression was significantly decreased in samples with methylated CDH1 promoter (p=0.048). These data suggest that E-cadherin could be relevant biomarker for early and specific diagnosis and may improve cervical cancer management.

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PS17.07

Epigenome-wide Association study of coffee and tea consumption

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Exposure to chemicals and toxic agents can alter DNA methylation. One common exposure in most human populations is coffee and tea consumption. Coffee has been suggested to play an important role in risk of disease in humans by suppressing tumour progression, decreasing inflammation and influencing estrogen metabolism.

We performed a genome-wide DNA methylation study for coffee and tea consumption in 718 individuals from the northern Sweden. DNA methylation was measured, in white blood cells, at more than 470,000 sites distributed throughout the genome.

We identified one genome-wide significant CpG site (cg12934382, P-value = 2.7x10-8) associated with coffee consumption, and no sites associated with tea. Cg12934382 is located close to the transcription-starting site of the Glutamate Receptor 2 gene (GRM2). Glutamate receptors are used as therapeutic targets for Parkinson's disease, and GRM2 has also been associated with Schizophrenia. Interestingly, it has been shown that high caffeine consumption is associated with increased symptoms in Schizophrenia patients and that hypermethylation of GRM2 decrease the risk of Schizophrenia. Oppositely, for neurodegenerative diseases such as Parkinson's and Alzheimer's disease, coffee consumption is associated with reduced risk.

In summary, we have identified that DNA methylation in GRM2 is associated with coffee consumption. The link between Glutamate receptor genes and diseases that have been linked to coffee consumption suggests that GRM2 might be mediating this effect. As a continuation we will replicate our findings in a set of independent study cohorts, and meta-analyse genome-wide methylation data from almost 3000 samples, as part of the EWAC consortium.

PM17.08

The CRC-predicting diet induced expression changes in normal colon mucosa

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Colorectal cancer (CRC) is the second most common cause of cancer related deaths in the Western world and interactions between genetic and environmental factors, including diet, play a critical role in its etiology. We conducted long-term feeding experiment in a mouse to address expression changes arising in normal colonic mucosa and their functional consequences as putative cancer-predisposing events available for early detection. Previously the expression of 94 growth-regulatory genes linked to human CRC and aber-

rant hypermethylation was studied for 5 weeks and 12 Months old Mlh1+/- mice analogous to human Lynch syndrome (LS) and wild type littermates, fed either by Western-style diet (WD) or control diet. Histologically normal proximal colonic mucosa, the predominant site of cancer formation in LS, exhibited a significant diet/genotype induced expression decrease in numerous CRC related tumor suppressor genes e.g. Cdh1, Dkk1, Slc5a8, and Socs1. The reduced expression was associated with age-related promoter hypermethylation.
Study continues with whole transcriptome RNA-sequencing to check the status of the previously found affected genes, affected pathways, and new CRC candidate genes in 18 and 21 Months old mice. The link from expression changes to cancer is studied from colonic tumors, from which the protein expression of susceptibility gene Mlh1 and beta-catenin was studied with immunohistochemistry. None of the tumors were found to be Mlh1 negative or to express nuclear beta-catenin. Therefore, the molecular reason behind tumorigenesis in our mouse series still remains to be elucidated. Yet, the role of WD in carcinogenesis is underscored by the fact that over 80 % of tumors were found in mice fed with WD.

PS17.09

Genomic/epigenomic defects associated with surprisingly detected parental consanguinity uncovered by molecular karyotyping with SNP arrays

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Parental consanguinity is generally accepted to be a risk factor for hereditary diseases. However, parents may not know about their consanguinity. As a result, clinical genetic evaluation is unable to provide a preliminary diagnosis for performing targeted molecular genetic diagnosis. Fortunately, there do exist whole-genome scanning techniques making possible to detect not only genomic but also epigenomic variations (i.e. segmental loss of heterozygosity or SLOH) for evaluating levels of consanguinity. Here, we have analyzed genomes of 188 children with intellectual disability, autism, epilepsy and/or congenital malformations using SNP/oligonucleotide molecular karyotyping (Affymetrix) and an original bioinformatic technology for addressing phenotypic, cellular and molecular consequences of genomic/epigenomic variations. SLOH, specific to clinical manifestations in these children, were found in 6 cases (3.2%). It is to note that parental consanguinity was not initially reported in all these cases. In one child, Meier-Gorlin syndrome was diagnosed. Other cases were also associated with hereditary diseases. Two cases of SLOH indirectly related to the phenotype were associated with microdeletions in 14q22.1 and 15q15.2, respectively. Parental consanguinity has long been considered as a source of knowledge about origins of hereditary diseases. In the present study, the possibility of genetic analyses of children born in consanguineous marriages has been extended by whole-genome and bioinformatic analyses. Actually, it has been shown that surprisingly detected consanguinity can be relatively common and molecular diagnosis using SNP arrays and bioinformatics can be useful for revealing of mechanisms of genetic and epigenetic diseases. Supported by Russian Science Foundation (Grant: №14-15-00411).

PM17.10

Identification of trisomy 21 specific biomarkers based on transcription level characteristics

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Non-invasiveprenatal diagnosis has been one of the most challenging fields in the past two decades. The discovery of fetal nucleic acids in the maternal circulation encouraged several groups to work on the identification of fetal specific biomarkers. In order to expand the
panel of fetal biomarkers, we targeted the whole transcriptome to identify differentially expressed genes (DEGs) among the trisomy 21 and normal fetuses from their mothers. The majority of the studies, working on biomarker discovery, focused on chromosome 21 but the phenotypic characteristics of Down syndrome (DS) suggest that can be related to genes located on other chromosomes as well. Expression microarrays covering the whole transcriptome were applied to four normal and two trisomy 21 chorionic villi (CVS) RNA samples together with their matching maternal RNA (white blood cells). Association studies of identified DEGs with Down syndrome phenotype were performed. Multiple trisomy 21 specific DEGs, 175 in number, were identified and found to be located across the whole genome. Nine of them were directly associated with the DS phenotype. Furthermore, many of the remaining DEGs were indirectly associated through pathological conditions occurring in DS. Findings of this study strongly support the hypothesis that DS phenotype is expressed from the disruption of the whole genome and not only chromosome 21 encoded genes. DEGs could eventually be used as fetal specific biomarkers for the identification of trisomy 21 from normal fetuses. Due to the transcription level variability among individuals, a large-scale study is needed in order to confirm our findings.

PS17.11

Epigenetic modifiers of cystic fibrosis

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Cystic Fibrosis (CF) is caused by mutations in the CFTR gene. T-lymphocytes from patients with CF are characterized by reduced expression of anti-inflammatory cytokines, such as IL-10 and elevated level of pro-inflammatory cytokines (e.g.IL-8). Epigenetic factors such as DNA methylation or histone modifications might contribute in cytokine dysregulation during CF. DNA methylation is catalyzed by DNA-methyltransferase enzymes (DNMTs).
In this study the correlations between levels of DNMT1, DNMT3a and IL-10 were investigated in T-lymphocytes derived from CF patients and healthy subjects. T-lymphocytes were isolated by ficoll gradient centrifugation with subsequent purification and cultured in RPMI 1640 medium supplemented with 5% L-glutamine and 10% FCS, with presence or absence of 5-azacytidine (5-AzaC; Sigma- Aldrich), a DNA methyltransferase inhibitor, for 24h. T-cells were activated with ionomycin (250 ng/ml) and PMA (20ng/ml). Levels of DNMT1 and DNMT3a were measured in nuclear extracts using DNMTs assay kits (Abcam, UK). IL-10 amount from supernatant of T-cell culture was quantified by ELISA kit (Abcam, UK).
We have found that T-lymphocytes from CF patients express elevated level of DNMT3a (40%), while no significant changes was observed at levels of DNMT1 compared to healthy subjects. In presence of the high dose of 5-AzaC (6μmol/L) the level of DNMT3a was decreased which was accompanied by an increased amount of IL-10.
These findings show the immunomodulatory effect of 5-azaC in CF T- lymphocytes. Since aberrant epigenetic modifications are potentially reversible epigenetic drugs might represent targets for Cystic Fibrosis therapy. Further work will involve evaluation of a promoter methylation status of cytokine genes in CF T- cells .

PM17.12

Epigenetic alterations in genes, connected to stress and toxicity, in the course of type 2 diabetes

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Type 2 diabetes (DM2) is a chronic metabolic disease resulting from genetic, epigenetic and environmental factors. Its pathogenesis and progression are related with chronic oxidative stress-an important mechanism for glucose toxicity.
The aim of our study was to define the epigenetic alterations in DM2. First, we have determined mRNA expression levels of methyl-CpG binding domain protein 2 (MBD2) in 16 patients and 12 controls. Then, we have analyzed DNA methylation status of 22 genes, connected to stress and toxicity, in four DNA pools of: 20 healthy controls; 20 initial DM2 patients; 20 patients with DM2 for less and 20 patients with DM2 for more than five years. The expression analysis and methylation status survey were performed by Real-Time PCR, preceded by reverse transcription and restriction with methylation-sensitive and methylation-dependent enzymes, respectively.
Our results show that MBD2 has increased expression in DM2 (more than 4 times). From the genes analyzed for promoter methylation, four show increasing methylated fraction with the course of DM2: BRCA1 (0% in healthy controls; 5.9% in initial DM2; 0.5% in DM2 for less than 5 years and 47.8% in DM2 for more than 5 years), CCND1 (0%, 13.6%, 14.7% and 34.1%, respectively), SCARA3 (3.5%, 8.1%, 20.2% and 23.3%, respectively) and Prdx2 (7.3%, 22.3%, 22.4% and 32%, respectively).

In conclusion, the increased MBD2 expression in DM2 seems to be related to DNA methylation. This involves genes for DNA repair and cell cycle regulation (BRCA1 and CCND1) and genes with antioxidant protective role (SCARA3 and Prdx2).
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PS17.13
Role of the genetic background in epigenetic response to phtalate exposure on a genome wide scale

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Di-(2-ethylhexyl)phtalate (DEHP) is a plasticizer with endocrine disrupting properties found ubiquitously in the environment. Studies in rodents and in humans revealed that it is an aggressive reproductive system toxicant.
The possible impact of DEHP exposure during pregnancy in mice was assessed on sperm count and on DNA methylation by MBD-sequencing in the F1 male generation using two different genetic backgrounds, C57BL6 and FVB mice. DEHP exposure induced a C57BL6 strain-dependent statistically significant reduction in sperm counts (p = 8.7x10-4) with no detectable impacts in the other strain tested (FVB mice, p= 0.9). In the sperm of DEHP exposed F1 mice, MBD-sequencing analysis taking in account the strain and the DEHP exposure revealed statistically significant DNA methylation enrichments in promoters of four genes: Tmem125, Piwil2, Fkbp1a and Smim8. Among them, Piwil2 was previously shown to be hyper-methylated in the testicular samples of human infertile males, whereas Fkbp1a stimulates sperm motility. Piwil2 and piwil4 were the two most significant and recurrent candidates identified in the approach targeting genes involved in reproduction according to Go terms. Transcription level of a subset of targets was tested by RT-qPCR on sperm RNA. Results showed that promoter methylation was globally inversely correlated with gene expression. Finally, functional genetic variations between both mice strains were localized on some of the targets identified by MBD-sequencing.
This study reveals an important strain-dependent impact of DEHP exposure during pregnancy on the production of sperm cells. It was associated with epigenetic dysregulation occurring in genes involved in the reproduction process.

PM17.14
ANALYSIS OF microRNA EXPRESSION WHEN PERFORMING EXERCISE TESTING STUDENT-ATHLETES

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We are analyzed the biochemical, immunological, echocardiography and electrocardiogram markers of the stress response when performing exercise testing student-athletes. The study of gene expression, biochemical and immunological analyses were performed to exercise testing and immediately afterwards. All subjects gave their written informed consent to the experiment. Created a collection of microRNAs from the blood using a set of reagents „RNeasy Protect Animal Blood Kit („Quigen, USA). Quantitative and qualitative assessment of selected microRNAs were performed on the 2200 TapeStation Instrument (Agilent technologies, USA) and „Qubit“ („Invitrogen, USA). Sequencing was performed using a set of reagents „Ion PGM™ Sequencing 300 Kit and a microchip „Ion 314™ Chip“ („Life technologies, USA). The data analysis sequencing was performed by aligning the nucleotide sequences against a reference genome HG19 using the resource Alignment v3.6.56201“, and generate the required file format (*.bam *.vcf) using Torrent Server according to the instructions, Torrent Suite 3.4.1“ („Life technologies, USA). Annotating and filtering of variants was performed using the Internet resource „Gene Talk‘ (http://www.gene-talk.de). So using the resource for data analysis NGS sequencing microRNA - omiRas were identified changes in the expression for hsa-mir-185 (differ before and after load). MicroRNA hsa-mir-185 is associated with the regulation of the following genes: PPARG, VEGFA. The products of these genes are associated with the physical performance. Obtained preliminary results allow us to speak about the role of microRNAs in the stress response of athletes on physical activity. However, requires verification of the received data using realtime PCR.

PS17.15
Analysis of histone modifications in Familial Mediterranean Fever patients

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Introduction: Familial Mediterranean Fever (FMF) mostly exhibiting autosomal recessive inheritance (1:1000 in Turkey) is the most common autoinflammatory disease. More than 200 FMF-associated variations have been reported in the MEFV gene. MEFV gene expression levels are generally low in FMF patients compared to healthy controls, and further decrease has been observed during attacks which appears to be associated with inflammation, Furthermore, methylation level of exon 2 of MEFV was found to be higher in FMF patients which reversely correlates with expression. In this study, in addition to the link between DNA and histone lysine methylation, we wanted to explore heterochromatin and euchromatin modifications in FMF patients during the attack and attack-free period in order to assess whether the modifications are related to inflammation.
Materials and Methods: This study included 10 FMF patients during the attack and attack-free period and 10 age-gender matched healthy controls. Modification-specific antibodies (trimethylation(me) of H3K9 and H3K4) are used for chromatin immunoprecipitation(ChIP) experiments to enrich modified regions in Peripheral Blood Mononuclear Cells (PBMCs) isolated from peripheral blood. ChIP experiment was combined with sequencing (ChIP-seq) and was performed on one Illumina Hiseq 2500 lane, 177,447,268 bases in total.
Results: We detected H3K4me3 modification on many genes mostly localized in chromosome 11, 12 and 16 which harbours candidate genes for inflammation.
Conclusions: Although bioinformatic analyses are still ongoing in depth, we currently searching promising candidates with H3K9me3 modification. Further studies will be conducted to determine whether these modifications are associated with MEFV and/or other components of inflammatory pathway.

PM17.16
Investigating the regulatory landscape of the human fibrinogen gene cluster

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Introduction: The coagulation factor fibrinogen is expressed and secreted by hepatocytes and its circulating concentration is associated with cardiovascular disease (CVD) risk. Three clustered genes (*FGA, FGB* and *FGG*) encode the three polypeptide components of fibrinogen. They are coordinately regulated and we previously identified two intergenic enhancer elements. Our aim is to uncover further regulatory elements and understand how local chromatin architecture influences fibrinogen expression.
Materials and Methods: A luciferase reporter assay in fibrinogen-expressing (HepG2) and non-expressing (HEK-293T) cells and a transgenic zebrafish assay were used to demonstrate liver enhancer activity for predicted regulatory sequences near the fibrinogen locus. Chromatin interactions will be assessed by chromatin conformation capture and their role in the regulation of fibrinogen expression studied using CRISPR-Cas9 editing.
Results: We identified two enhancers flanking the fibrinogen locus. Each sequence enhances reporter gene activity in HepG2 cells irrespective of orientation and position with respect to a fibrinogen promoter, and one drives liver transgene expression in zebrafish larvae. ENCODE chromatin immunoprecipitation-sequencing data demonstrate that each enhancer is bordered by CTCF and RAD21 interaction sites, suggesting that the extremities of the gene cluster may be looped together. We are investigating the functional importance of these potential interactions.
Conclusions: The fibrinogen locus is flanked by liver enhancers and may adopt a looped conformation. Demonstrating functional importance of this looping in the regulation of fibrinogen expression could link chromatin interactions to circulating fibrinogen levels, and ultimately to CVD risk.
This work is supported by a Swiss National Science Foundation grant to MNA (#152633).

PS17.17

Reactivation of the FMR1 gene in fragile X lymphoblastoid cells by 5-aza-2-deoxycytydine does not cause random genomic DNA demethylation.

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Fragile X syndrome (FXS) is caused by CGG expansion over 200 repeats at the 5' UTR of the FMR1 gene and subsequent DNA methylation of both the expanded sequence and the CpGs of the promoter region. This epigenetic change causes transcriptional silencing of the gene. We have previously demonstrated that 5-aza-2-deoxycytydine (5-azadC) treatment of FXS lymphoblastoid cells reactivates the silent gene, allowing CpG sites demethylation, increased acetylation of histones H3 and H4 and methylation of lysine 4 on histone 3. Recently we observed that reactivation of FMR1 transcription is long lasting, up to a month after a 7-days treatment with 5-azadC, and that maximum level of transcription are reached 10-15 days after 5-azadC last administration. In order to check the specificity of the 5-azadC-induced DNA demethylation, we performed bisulphite sequencing of the entire methylation boundary upstream the FMR1 promoter region, which is preserved in WT cells [Naumann et al., 2009]. We did not observe any modification of the methylation boundary after treatment. Furthermore, methylation analysis by MS-MLPA of PWS/AS and BWS/SRS loci demonstrated that 5-azadC treatment has no demethylating effect on these regions. Methylation analysis through Infinium 450K (Illumina) revealed no significant changes in differentially methylated regions after treatment. Taken together these data show that 5-azadC has a long lasting reactivating effect on the mutant FMR1 gene and that its demethylating effect on genomic DNA is not random but rather restricted to specific regions. These findings may open new perspectives for a drug-based epigenetic therapy of FXS.

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PM17.18

FSHD 1 and 2 testing - a clinical diagnostic service perspective

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FSHD (affecting ~1 in 20,000 individuals) is an autosomal dominant epigenetically regulated disorder, with characteristic pattern of progressive muscle involvement commencing in the face and shoulder-girdle. Two clinically indistinguishable forms differ in molecular basis of epigenetic regulation, but are underpinned by hypomethylation of 4q35, causing aberrant DUX4 expression (toxic transcription factor). FSHD1 (OMIM158900) (95% cases) caused by contraction of D4Z4 repeats, results in allele-specific hypomethylation. FSHD2 (OMIM158901) (~3% of cases), contraction independent, is caused by mutations in SMCHD1, encoding a chromatin-modulating enzyme, resulting in global hypomethylation and chromatin relaxation of D4Z4 arrays. A permissive haplotype at 4q35 is required for clinical expression of FSHD1 and 2, necessitating digenic inheritance for FSHD2.

BGL provides a UKGTN specialist diagnostic service for FSHD processing >500 UK/international referrals annually. Clinically typical (assessed by clinical proforma) deletion negative patients (4.8% referrals) are tested for FSHD2 by 4q35 methylation quantification (pyrosequencing) followed by SMCHD1 sequencing of hypomethylated patients.

A diagnosis of FSHD2 has been confirmed in 15/45 cases, with 12/15 novelSMCHD1 mutations (the remainder are deletions of intron/exon 25 boundary, the first 'hotspot' identified in SMCHD1). We have compared the clinical severity of FSHD2 and age/sex matched FSHD1 patients.

FSHD2 shows digenic inheritance with risk to offspring between 25 and 50%, depending on the haplotypes of the wider family. Family testing has been undertaken for 3 cases.

We present the clinical and genetic data of the FSHD2 cohort, and interesting cases highlighting the clinical utility of genetic testing, and the complexity of counselling family risks associated with digenic disease.

PS17.19

Integration of the PPP1R26P1 human pseudogene sequence does not induce imprinting of Rb1 in the mouse

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The human RB1 gene is imprinted, carrying a differential methylation mark at an intronic CpG island (CpG85) on the maternal chromosome and showing biased expression of full-length RB1 in favor of the maternal allele. On the unmethylated paternal allele, CpG85 serves as promoter for an alternative RB1 transcript, which starts at a novel exon 2B and possibly interferes with expression of the RB1 gene from the main promoter. CpG85 is part of the PPP1R26P1 pseudogene, which integrated into intron 2 of the RB1 gene by retrotransposition. To study if integration of a pseudogene sequence per se is sufficient to induce differential methylation and imprinting, we generated a knock-in mouse, carrying human PPP1R26P1 in intron 2 of the mouse Rb1 gene.

Using next-generation bisulfite amplicon sequencing, we analyzed acquisition of DNA methylation at CpG85 in several tissues in male and female heterozygous mice after either maternal or paternal transmission of PPP1R26P1. We observed a tissue-specific pattern of DNA methylation with high levels in neuroectodermal tissue, like brain and eye, but low levels in all other tissues tested. This pattern was observed independent of parental transmission, indicating that CpG85 in the human pseudogene does not act as stable gametic DMR in the mouse. Preliminary analysis of CpG85 methylation in germ cells showed that it is unmethylated in sperm and in oocytes. This indicates that the integration of a foreign pseudogene sequence is not sufficient for establishment of differential methylation at CpG85 in the mouse germline.

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PM17.20

Prediction of imprinted genes based on the genome-wide methylation analysis

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Genomic imprinting is an epigenetic gene-marking phenomenon that occurs in the germline and results in monoallelic expression according to parental origin. Our hypothesis is that imprinted genes in humans can be predicted by the methylation level of CpG sites within that gene region. We also expect that the variability of beta values is smaller within imprinted genes as they have more semi methylated probes and therefore more medium beta values.

For this study 17 tissues from 4 individuals were collected during autopsy. DNA methylation analysis was performed with the Illumina Infinium HumanMethylation450 BeadChip in Estonian Genome Centre.

As a result, all imprinted genes (n=77) demonstrated less variability in the methylation level ($p < 0.01$) across all 17 tissues when compared to non-imprinted genes. We visualized the CpG patterns of known imprinted genes across all tissues. The visualized CpG patterns confirmed tissue-specific nature of imprinted genes. We found a tissue-specific switch of imprinted status across somatic tissues for two genes - MKRN3 and KCNQ1DN. CpG patterns also showed expression-specific patterns of genes, such as bi-allelic, monoallelic or combined expression. We also found that methylation pattern remains stable not depending on a gene being expressed or not. Based on this approach, we selected 50 potentially imprinted genes for further studies, 2 of which were already known as imprinted and 1 was predicted by another study.

Our method can be used as an efficient tool to clarify the nature of the already established imprinted genes as well as to discover new imprinted genes across the whole human genome.

PS17.21

Mining novel candidate imprinted genes by using high resolution DNA methylation microarrays in multiple human tissues

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Different strategies have been recently applied in the search for novel imprinted genes (IG) in humans, either focusing on monoallelic expression or epigenetic signature. Aiming at revealing novel IGs, we generated genomic

methylation data for 67 samples from six different human tissues (leukocyte, brain, liver, breast, melanocyte, chorionic villus), using the Illumina HM450K platform. CpG sites mapped at gene promoters harboring CpG islands in the beta-value range of 0.38-0.65 were applied to call hemimethylated CpG sites potentially associated with IGs. CpGs common to at least three different tissues were selected. About 30% of known IGs were recovered, and 170 candidate IGs were identified. Among these, 27 were shown to have at least three CpG probes shared by three or more tissues. Differentially methylated CpGs have been previously reported for seven of them, thus supporting the hypothesis of these genes being imprinted: PLEC, PTCHD3, ZNF331, KIAA2013, SYCE1, HTR5A and ZNF232. Among our 27 top candidates, PLEC, TONSL and VPS28 are neighbor genes mapped to a 665 Kb segment at 8q24.3, and might constitute a novel imprinted cluster. Interestingly, 39 members of protocadherin (PCDH) clusters on 5q31.3 were found to have hemimethylated CpGs at promoter regions in multiple human tissues; Pcdh non-imprinted monoallelic and randomic isoform expression have been previously demonstrated in mice, and this might be the case in human. Our approach based on the methylation pattern in multiple tissues was successful to reveal hemimethylated CpG sites potentially associated with novel IGs or non-imprinted monoallelic expressed genes in humans.
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PM17.22
A retrospective analysis of the prevalence of imprinting disorders in Estonia

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Introduction: Imprinting disorders (IDs) are a group of rare congenital diseases affecting growth, development and metabolism. The cause of IDs is an aberrant expression of imprinted genes due to genetic or epigenetic abnormalities. At present, there are eight clinically well-recognized IDs. Because of high variability of clinical phenotype and molecular alterations, the exact prevalence of IDs is not known.
Methods: In this study we retrospectively reviewed records of all Estonian patients with molecularly confirmed ID diagnosis during the period 1998-2014.
Results: A total of 52 individuals with IDs were identified. 46% (24) of them had Prader-Willi syndrome (PWS), 23% (12) Angelman syndrome (AS), 13%(7) Beckwith-Wiedemann syndrome (BWS), 12% (6) Silver-Russell syndrome (SRS), 2% (1) Temple syndrome (TS), 2% (1) pseudohypoparathyroidism Ib (PHP-Ib) and 2% (1) transient neonatal diabetes mellitus (TNDM). No cases of Kagami-Ogata syndrome have been found. The age of diagnosis varied between 0.03 and 83 years. All cases of TS, PHP-Ib, TNDM and the most cases of SRS and BWS were diagnosed in the last 4 years due to new diagnostic tests. During the period 2004-2014 birth prevalence of PWS in Estonia was 1/13,634 live births (~1/18,000 worldwide), AS 1/32,722 (~1/16,000), BWS 1/40,903 (~1/13,000) and SRS 1/54,537 (~1/70,000). Thus the total prevalence of IDs in Estonia is 4/100,000.
Conclusions: The introduction of new diagnostic methods allows todiscover cases of rare and atypical IDs. Further studies are needed to determine the exact prevalence and etiological mechanisms of IDs. This work was supported by the Estonian Research Council grant PUT355.

PS17.23
Strategies to correct the epigenetic path KDM5C-H3K4me3 damaged in XLID/Epilepsy diseases

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Mis-steps in histone methylation-demethylation rounds have been directly involved in several forms of Intellectual Disability (ID) with Epilepsy. Lysine-specific demethylase 5C (KDM5C) is an X-linked gene which encodes a chromatin JmjC eraser with H3K4me2/3 demethylase activity. It is mutated in a spectrum of XLID and/or malignant Epilepsy and functions as a transcriptional repressor interacting with REST/NSRF, a master epigenetic hub critical for transition of neural progenitors to neurons. Noteworthy, a defective KDM5C-H3K4me3 path has been found in association with a mis-regulation of XLID/Epilepsy effector genes.
We report here methods to correct KDM5C fault by testing epi-drugs and RNA-based technology to target KDM5C-H3K4me3 axis. To this end, we used neuronally-differentiated Arx KO/Kdm5c-depleted ES cells, which show GABAergic abnormalities in association with a global increase of H3K4me3 signal. We have undertaken an in vitro analysis of a number chromatin compounds aiming of modifying the accessibility of the KDM5C transcription machinery. A strong up-regulation of Kdm5C/KDM5C has been obtained in presence of an inhibitor of HDAC at different points of in vitro differentiation, both in WT and Kdm5C-defective cells.
In addition, we generated synthetic lncRNAs antisense to KDM5C. In transfected cell lines, HEK293T and P19, we obtained increased levels of endogenous KDM5C protein. Given the H3K4 demethylase activity of KDM5C, we therefore tested the level of H3K4me3 and verified that the increase amount of KDM5C inversely correlates with a decrease in H3K4me3 signal. However, the snapshot of this captivating stratagems may become more well-defined once we will decipher the downstream effect of KDM5C upregulation in Arx KO/Kdm5C-depleted ES cell line.

PM17.24
Key regulatory transcription factors shaping the DNA methylome of breast cancer molecular subtypes

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Lowly methylated regions (LMRs), non-CpG island *loci* that usually contain specific transcription factor (TF) binding sites, have been suggested to act as regulatory elements that define cellular identity. To date, LMRs have not been reported in breast cancer (BC). In this study, we aimed to identify the key subtype-specific TFs that shape the breast cancer methylome. We selected subtype-specific LMRs using whole bisulfitome data available at TCGA. Differentially methylated regions (DMRs) within the BC subtype-specific LMRs were selected by comparing tumors and normal tissues in a larger TCGA cohort assessed by Illumina 450K arrays. Finally, we performed TF enrichment analyses in the identified DMRs and calculated Pearson's correlations between the methylation levels of the *loci* presenting subtype-specific TF motifs and the expression of the nearest genes. We found 4,409 and 4,711 differentially hypomethylated positions for basal and luminal subtypes, respectively, among which 1,062 positions were shared by both subtypes. These positions could be grouped into 1,185 and 1,358 DMRs, respectively. In addition, basal LMRs were depleted on *FOXD3* and enriched on *EBF1* motifs, which had previously been associated to basal BC. The methylation levels of the regions containing *EBF1* motifs showed a strong negative correlation with the expression of 719 genes, including *BTS2*, *CD74*, *CDCP1* and *SLPI*, putative oncogenes known to be specific for basal type and/or poor outcome BC cases. Together, our results establish a link between master TFs and cancer-specific DNA methylation profiles and highlight our workflow as a powerful strategy in the search of subtype-specific therapeutic targets.

PS17.25
Analysis of a potential interaction between the WNT10A- and the EBF1-risk loci for male pattern baldness

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Molecular genetic studies have identified at total of twelve genetic risk loci for male-pattern baldness (MPB). Among them two loci on chromosomes 2q35 and 5q33.3 located intronically within WNT10A and EBF1, respectively. Expression analyses indicated an allele-specific regulatory effect of the lead-SNP rs7349332 at the 2q35-locus on WNT10A-expression (Heilmann et al, 2013). Database research revealed that rs7349332 is in strong linkage disequilibrium with rs3856551. Interestingly, this variant is located within a binding site for the transcription factor EBF1, which suggests a potential functional interaction between the two loci. Here, we hypothesized the MPB-risk variant rs3856551 causes an allele-specific difference in the binding affinity of EBF1 to its target site at 2q35 that result in the observed differences in WNT10A-mRNA-expression.
To functionally prove this interaction, we performed in vitro luciferase assays in human keratinocytes by co-transfecting a luciferase construct containing the computationally predicted WNT10A promoter sequence, the 2q35-EBF1 binding site with either the C- or the T-allele for rs3856551, and an EBF1-expression vector. Our experiments indicate that EBF1 is able to activate the WNT10A promoter and point towards a significantly weaker activation for the rs3856551 C-allele. Our results therefore suggest a so far unknown functional interaction between the two MPB risk loci on 2q35 and 5q33.3. Additional experiments are ongoing to further prove this functional interaction. This may eventually help to elucidate the underlying molecular

mechanisms at these two MPB risk loci.

PM17.26

An investigation into the variation of mitochondrial methylation across different regions of post-mortem human brain.

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Mitochondrial dysfunction has been implicated in Alzheimer's disease. We recently demonstrated that epigenetic dysfunction is seen in the nuclear genome in Alzheimer's disease brain and blood. Mitochondria are a unique cellular organelle in that they possess their own unique genome of ~16kb and encode for 37 genes. We hypothesise that mitochondrial dysfunction in Alzheimer's disease may be driven by epigenetic changes in the mitochondrial genome. As no study has previously investigated this phenomenon in human post-mortem tissue, we investigated whether differences can be seen in mitochondrial DNA methylation between different anatomical regions of the brain using sequencing approaches. We identified 16 significantly differentially methylated regions (DMRs) ($p<0.05$) in 9 of the 37 mitochondrial-encoded genes between the cortex and the cerebellum. As over evolution, regions of the mitochondrial genome have inserted themselves into the nuclear genome (nuclear-mitochondrial pseudogenes (NUMTs)) we decided to bioinformatically account for these in our analyses, leading to dramatic changes in DMR calling (13 from 9 genes). This data highlights the importance to correct for NUMTs not only in mitochondrial genetic studies but also in epigenetic studies. Further, the data is the first to our knowledge to identify DMRs using these methods in the mitochondrial epigenome between different brain regions.

PS17.27

DNA methylome profiling of human tissues reveals global and tissue-specific methylation patterns

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Background
DNA epigenetic modifications, such as methylation, are important regulators of tissue differentiation, contributing to processes of both development and cancer. Profiling the tissue-specific DNA methylome patterns will provide novel insights into normal and pathogenic mechanisms, as well as help in future epigenetic therapies. In this study, 17 somatic tissues from four autopsied humans were subjected to functional genome analysis using Illumina's Infinium HumanMethylation450 BeadChips that cover above 486,000 CpG sites.
Results
Only 2% of the CpGs analyzed are hypermethylated in all 17 tissue specimens; these permanently methylated CpG sites are located predominantly in gene-body regions. In contrast, 15% of the CpGs are hypomethylated in all specimens and are primarily located in regions proximal to transcription start sites. A vast number of tissue-specific differentially methylated regions are identified and considered likely mediators of tissue-specific gene regulatory mechanisms since the hypomethylated regions are closely related to known functions of the corresponding tissue. Finally, a clear inverse correlation is observed between promoter methylation within CpG islands and gene expression data obtained from publicly available databases.
Conclusions
This genome-wide methylation profiling study identified tissue-specific differentially methylated regions in 17 human somatic tissues. Many of the genes corresponding to these differentially methylated regions contribute to tissue-specific functions. Future studies may use these data as a reference to identify markers of perturbed differentiation and disease-related pathogenic mechanisms.
This study was recently published in the Genome Biology magasine,

PM17.28

Combined overexpression of 3 microRNAs leading to autophagy dysfunction as a new pathophysiological mechanism in Hutchinson-Gilford Progeria Syndrome

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The Hutchinson-Gilford Progeria Syndrome (HGPS) is a rare genetic disease with accelerated aging, due to the accumulation in nucleus of a toxic protein called progerin, leading to abnormal gene expression and potential microRNA (miRNA) deregulation. To evaluate the role of miRNAs in HGPS, we conducted an in vitro miRNome analysis on dermal fibroblasts by studying 5 patients and 5 healthy individuals of different ages at early (P12+/-2) and late passages (P22+/-2). We identified 29 deregulated microRNAs in more than 50% of patients (15 overexpressed, 14 underexpressed) with different deregulation profiles depending on their age and passage in vitro. We identified 4 interesting potential targeted pathways linked to aging/Progeria: cell cycle and proliferation, senescence, inflammation and autophagy for which 3 microRNAs target central actors of this pathway. No significant difference between patients and controls was detected for 3 autophagy makers on western blotting. However, using flow cytometry, allowing quantification of autophagy level cell by cell, we observed in a 14 yo patient exhibiting the most miRNA deregulated profile, a majority of cells having no autophagy. Our hypothesis is that the overexpression of the 3 autophagy inhibitor miRNAs act as a "brake" on autophagy, leading to a decrease of progerin degradation, and finally to a pathophysiological vicious cycle. We will now confirm this hypothesis by transfecting antagomirs on cellular model. We will also evaluate this mechanism in our HGPS LAKI mouse model and in the context of physiological aging, during which progerin is also produced.

PS17.29

Methylome analysis for spina bifida identified hypomethylation of multiple genetic loci and distinct pathways linked to neural tube defects

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Introduction: Neural tube defects (NTDs) are severe congenital malformations that arise from the failure of neurulation during embryonic development. Though family- and population-based studies have confirmed a genetic component, responsible genes for NTDs in humans are still largely unknown. Based on the hypothesis that folic acid prevents NTDs by stimulating methylation reactions, epigenetic factors, such as DNA methylation, are predicted to be involved in NTDs.
Materials and Methods: Methylome analysis using the 450K array was performed using leukocyte DNA of 10 myelomeningocele (MMC) patients and 6 controls. A case-control validation study with the Sequenom Epityper analyzed selected candidate loci with altered methylation in larger cohorts (83 MMC and 30 controls). Gene overexpression studies and phenotype analysis in a zebrafish model of neural tube formation are ongoing.
Results: First, analysis of methylome data using a candidate pathway approach identified *HOXB7* as risk factor for NTDs. Interestingly, *HOXB7* hypomethylation was confirmed in the validation study and *HOXB7* overexpression in zebrafish resulted in NTDs. Secondly, genome wide analysis of the methylome data showed significant hypomethylation of 7 candidate loci that could also be validated with the EpiTYPER. Third, gene prioritization analysis based on functional protein associations, discovered interesting candidate loci.
Conclusions: Our data implicate DNA hypomethylation as risk factor for NTDs with at least 8 novel candidate genes underlying NTDs.

PM17.30

DNA 5-Hydroxymethylation in human adipose tissue differs between subcutaneous and visceral adipose tissue depots

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Background: Epigenetic mechanisms such as DNA methylation at the 5-carbon of cytosine (5-mC) were shown to have tissue specific patterns and play a role in post-transcriptional regulation of gene expression. While 5-mC is well studied, the discovery of a stable intermediate during DNA de-methylation (5-hydroxymethylcytosin (5-hmC)) raises questions about its function and distribution. The aim of this study was to test whether 5-hmC exists in human subcutaneous (SAT) and visceral adipose tissue (VAT) and correlates with anthropometric and metabolic parameters.

Materials and methods: We used a sample set of 81 subjects (42 women and 39 men) to measure the % 5-hmC content in both SAT and VAT by using ELISA technology. To test for associations with anthropometric and metabolic parameters we used paired students t-tests, bivariate correlation analyses and linear regression models.
Results: We observed an average 5-hmC content of 0.47 % ± 0.093 in SAT, while VAT (0.51% ± 0.122) is significantly higher hydroxymethylated (P = 0.005). In the total cohort we observed a positive association of % 5-hmC in VAT with age (P = 0.034). Furthermore, we identified a significantly negative relationship between % 5-hmC in VAT and LDL cholesterol levels which withstands adjustment for covariates and remains significant after correction for multiple testing (P = 0.008).
Conclusion: Our data suggest adipose tissue depot specific 5-hmC levels with significantly higher levels in VAT.
Grant References: DDG; DDS; IFB AdiposityDiseases; EFSD; BMBF; Competence network for Obesity; SFB1052/1

PS17.31

Hyperactive Rho-kinase signalling due to Oligophrenin-1 loss of function induces nuclear import of class IIa Histone Deacetylase (HDAC7) and downregulation of orphan nuclear factor NR4A1.

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Rho-kinase (ROCK) has been well documented to play a key role in RhoA-induced actin remodeling. ROCK activation results in Myosin light chain (MLC) phosphorylation either by direct action on MLC kinase (MLCK) or by inhibition of MLC phosphatase (MLCP), modulating actin-myosin contraction. We found that inhibition of the ROCK pathway in induced pluripotent stem cells, leads to nuclear export of HDAC7 and transcriptional activation of the orphan nuclear receptor NR4A1 while in cells with constitutive ROCK hyperactivity due to loss of function of the RhoGTPase activating protein Oligophrenin-1 (OPHN1), the orphan nuclear receptor NR4A1 is downregulated. Our study identify a new target of ROCK signaling via Myosin Phosphatase Subunit (MYPT1) and Histone Deacetylase (HDAC7) at the nuclear level and provide new insights in the cellular functions of ROCK.

PM17.32

Cardiovascular GWAS PEAR1 SNP stimulates its intronic enhancer activity via DNA methylation

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Platelet Endothelial Aggregation Receptor-1 (PEAR1) is a cell-cell contact protein mainly expressed in platelets and endothelial cells. A common genetic variant in PEAR1 (rs12041331) influenced platelet aggregation in aspirin-treated high-risk CVD patients. Reduced PEAR1 expression and decreased blood platelet function have been associated with the minor A-allele of this SNP (G>A) that is located in intron 1 of PEAR1. We aimed to unravel the mechanism by which this non-coding SNP leads to changes in PEAR1 expression.
Luciferase assays in HEK293 cells transfected with the pGL3-promoter vector showed that the intronic region containing the G allele possesses significantly higher transcriptional activity compared to the A allele ($p<0.05$). The G allele induces a potential CpG unit in this intronic region that can be methylated. Bisulfite-sequencing of rs12041331 in leukocyte DNA samples from 10 G/G healthy individuals showed that the cytosine at this position is indeed fully methylated. EMSA experiments showed that the rs12041331 region (chr1:156869698-156869731) containing the methylated G allele strongly interacts with EAHY926 endothelial cells nuclear proteins compared to the presence of the non-methylated G allele or the A allele.
ChIP experiments using DNA from EAHY926 endothelial cells and CHRF megakaryocytic (platelet progenitor) cells showed a significant enrichment of the active enhancer-specific histone mark H3K4me1 for the rs12041331 region.
In conclusion, we have found evidence that the rs12041331 SNP containing intronic PEAR1 region functions as transcriptional enhancer. Moreover, the methylated G-allele is associated with increased enhancer activity, which could explain the association of this SNP with changes in platelet reactivity important for CVD.

PS17.33

Transcriptome analysis by RNA-seq in Bloom's syndrome reveals genes associated to regulation of immune pathways.

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Transcriptome analysis by next-generation sequencing (RNA-seq) allows a deep investigation of RNA expression and could be a powerful approach to identify networks of genes that play a role in disease. We investigate the transcriptome using HiSeq 2500 platform (Illumina) followed by differential gene expression analysis of samples derived from two patients affected by Bloom's syndrome and three unaffected controls. The raw data were processed and analyzed using two different methodologies: Bowtie2 and EdgeR programs with filtering and the Rsubread and DESeq2 programs without filtering. The genes found differentially expressed underwent a functional enrichment analysis by DAVID. The analysis showed a highly complex transcriptional setting with upregulated differentially expressed genes associated to activation and regulation of immune pathways. Our results suggest that immune responses can be most active in these patients in order to compensate for a probable post transcriptional imbalance leading to genomic instability in patients with Bloom's Syndrome.
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PM17.34

Altered miRNAs expression profiles in peripheral blood cells from Systemic Sclerosis patients by small RNA deep sequencing

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Systemic Sclerosis (SSc) is a complex autoimmune disease of unknown etiology. The SSc is characterized by vascular dysfunction and excessive accumulation of extracellular matrix, resulting in progressive fibrosis leading to the failure of the affected organs. Previous studies identified specific deregulation of miRNAs involved in autoimmune, vascular or fibrotic processes in skin samples from patients with SSc. Moreover, a specific miRNA profile is expected to be found also in blood samples from SSc patients that might be useful as biomarkers for diagnosis prognosis or disease severity. We obtained the transcriptional profiling of 17 PBMC from SS patients and 10 control samples using the methodology of massive sequencing of miRNAs by the small RNA-Seq Illumina technology. Illumina sequencing unaligned reads data were aligned to the human subset of miRBase21 with novoalign (novocraftv3). Known miRNAs had a broad range of expression level. There were aprox. 330 mature miRNAs with more than 10 sequence reads and 32 miRNAs with more than 10.000 sequence reads. Differential expression analysis revealed 17 miRNAs significantly different between peripheral blood samples from SSc patients comparing to controls. 12 of them were found downregulated in SSc samples. Among these miRNAs, we detected miR-29a, miR-29b, miR-150, let-7g, miR-145, and miR-31 which were also found deregulated in the skin of patients with SSc. Other miRNAs not yet associated with the disease were also found. Analysis of blood cells miRNA expression may be useful to characterize the differential expression affecting immune cells, and to identify novel differentially expressed miRNAs associated with SSc.
Grants: TPY1250/12 and PI14CIII/00070.

PS17.35

Genome-wide landscape of genetic variation with a functional impact on predicted binding activity of transcription factor binding sites

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Introduction: Binding of transcription factors (TFs) to specific DNA sequences is essential for regulating the spatial and temporal expression of

genes. Approximately 10 % of known genes code for TFs, and with the FANTOM5 project TF expression profiles have been firmly linked to gene expression profiles in multiple tissues and cell types. Studies indicate that common variants have low likelihood of falling within transcription factor binding sites (TFBSs), indicating purifying selection. Though tools like FunSeq2 have been developed that can predict breaking or conserving effects of variants on TFBSs, the genome-wide impact of known variants on TFBS activity remain largely unexplored.
Materials and Methods: A high quality catalogue of human variation obtained by sequencing, including more than 80 million variants, from 2504 individuals and 26 populations, available from the 1000genomes project were used for the analysis. Characteristics of genetic variants and their effects of TF binding scores, calculated using position weight matrices, were annotated using a custom script interacting with the ENSEMBL database.
Results: We defined different classes of genetic variants, based on their impact on binding scores, and identify the most functionally relevant category, by performing enrichment analyses with variant sets from genome-wide association studies and different categories of diseases.
Discussion: Our work provides a more comprehensive genome-wide picture of the impact of genetic variation on TFBSs. The identification of rare variants, more likely to play a role in different pathologies, through the activity of TFs, will help better annotation and understanding of the results of future sequencing studies.

PS17.37
Exposure to ultraviolet radiation B has an acute effect on gene expression in blood

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Introduction: Ultraviolet B (UVB) radiation, representing 5% of total solar UVR, is responsible for vitamin D production and suppresses the immune system locally and systemically, with potential beneficial effects for autoimmune diseases. We studied the effects of UVB on the whole blood transcriptome at different time points after exposure.
Material and Methods: Nine Caucasian males with skin type II and mean age 25 years were exposed to sub-erythemal UVB [whole body exposure of 2.5 standard erythema doses (SED)]. Blood samples were collected in PAXGene tubes before exposure (0h) and after 6h, 24h and 48h of exposure. RNA was extracted and mRNA libraries were prepared with the Illumina TruSeq mRNA Sample Preparation kit and sequenced in a HiSeq2000 equipment (100 nt, single reads).
Results: An average of 17.3 M reads per sample (SD: 4.6, min: 9.8M, max: 28.7M) were mapped on the human genome and approximately 22% of them represented globin genes. At 6h post exposure, 9 downregulated and 1 upregulated gene were detected at a False Discovery Rate (FDR) of 5%. The most relevant genes are: FLT3, a class III tyrosine kinase regulating hematopoiesis and involved in acute myeloid and lymphoblastic leukemia; CPM, a carboxypeptidase that participates in monocyte and macrophage differentiation; and FKBP5, a member of the immunophilin family, which plays a role in immunoregulation. No significant changes were identified at 24h or 48h, indicating a short-lived acute effect.
Conclusions: Exposure to UVB decreases expression of genes involved in immunoregulation in blood at 6h post exposure.

PS18.01
Admixture Analyses of phenotypes related to the metabolic syndrome in a Brazilian population

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Background: We conducted admixture analyses of metabolic syndrome in the Baependi family study from the state of Minas Gerais, Brazil to better understand the basis of ethnic differences in the metabolic syndrome. Methods: The Baependi family study consists of 80 families and 1,109 subjects with complete clinical information and genotype data from Affymetrix 6.0 SNP chip. Genome-wide admixture analysis was performed to test whether local ancestry in this data was associated with any of the phenotypes in the metabolic syndrome using a mixed linear model adjusted for age, sex, principal components 1 and 2 and taking into account the family structure. Results: Admixture analysis was performed using PCAdmix, a principal components based algorithm for determining ancestry along each chromosome from a high-density genome-wide set of phased single nucleotide polymorphism (SNP) genotypes of admixed individuals. It returns a local ancestry estimate for haplotypes for each reference population. We used HapMap Phase 3 CEU and YRI samples and the HGDP Native American (NA) samples as the reference for PCAdmix. We observed an average local ancestry to be 0.69/ 0.13/ 0.18 across the genome for CEU/ YRI/ NA, respectively. We observed a peak on chr2p due to African ancestry for systolic blood pressure, on chr3 due to Native American ancestry for diastolic blood pressure, and on chrs 2q and5q due to African ancestry for truncal obesity. Conclusions: In summary, by performing association analysis using local ancestry help to understand the ethnic differences in phenotypes related to the metabolic syndrome better than the standard association analysis.

PM18.02
A candidate gene association study identifies DAPL1 as a female-specific susceptibility locus for age-related macular degeneration (AMD)

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Age-related macular degeneration (AMD) is the leading cause of blindness among white caucasians over the age of 50 years with a prevalence rate expected to increase markedly with an anticipated increase in the life span of the world population. To further expand our knowledge of the genetic architecture of the disease, we pursued a candidate gene approach assessing 25 genes and a total of 109 variants. Of these, synonymous single nucleotide polymorphism (SNP) rs17810398 located in DAPL1 (death associated protein-like 1) was found to be associated with AMD in a joint analysis of 3,229 cases and 2,835 controls from five studies (combined P = 1.15×10-6, OR=1.332 [1.187-1.496]). This association was characterised by a highly significant sex difference (Pdiff = 0.0032) in that it was clearly confined to females with genome wide significance (PADJ = 2.62×10-8, OR = 1.541 [1.324-1.796]; males: PADJ = 0.382, OR = 1.084 [0.905-1.298]). By targeted resequencing of risk and non-risk associated haplotypes in the DAPL1 locus, we identified additional potentially functional risk variants, namely a common 897bp deletion and a SNP predicted to affect a putative binding site of an exonic splicing enhancer. We show that the risk haplotype correlates with a reduced retinal transcript level of two, less frequent, non-canoncical DAPL1 isoforms. DAPL1 plays a role in epithelial differentiation and may be involved in apoptotic processes thereby suggesting a possible novel pathway in AMD pathogenesis.

PS18.03
Further analysis of human salivary and pancreatic amylase gene CNVs

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The human amylase gene cluster is a highly copy number variable region. Salivary amylase (*AMY1*) copy number variation (CNV) has been correlated with the protein level, and high *AMY1* copy number appears to result from adaptation to a high starch diet[1]. Additionally, *AMY1* CNV has also recently been associated with body mass index (BMI)[2]. In the present study, we developed two PCR ratio assays to explore the pancreatic amylase (*AMY2*) variation, and a paralogue ratio test (PRT) for measuring *AMY1* copy number. Combining our results with previous PRTs and read depth analysis, we observed variation in the *AMY2* genes in the form of deletion or duplication of *AMY2A* and duplication of *AMY2A/AMY2B*.The frequency of these variations was different between the studied populations, in most of which *AMY2* CNV showed associations with *AMY1* copy number. The *AMY2* genes exhibit less extensive variation than the salivary amylase genes; most individuals have two copies of *AMY2A* and *AMY2B*. However, segregation analysis in African family trios revealed new arrangements of the pancreatic amylase genes. Some haplotypes harbour higher order duplication of *AMY2A/AMY2B*, extending to quintuplication of *AMY2A/AMY2B*. Furthermore, our results suggest that the PRT we developed is a simple, accurate and high throughput method for measuring *AMY1* copy number in a large set of samples.

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PM18.04

Apolipoprotein E gene polymorphisms and lipid levels in patients with T1D

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Introduction: Apolipoprotein E (APOE) genetic variants have a major influence on lipid metabolism and represent a risk factor for development of cardiovascular disease in type 1 diabetes (T1D) patients. The c.334T>C (rs429358) and c.472C>T (rs7412) genetic variants in gene encoding APOE determine 3 major alleles: e2, e3, e4 and 6 corresponding genotype combinations: e2/e2, e2/e3, e2/e4, e3/e3, e3/e4, e4/e4. The aim of this study was to determine the influence of APOE genetic variants on serum concentrations of total cholesterol (TC) and triglycerides (TG) in T1D patients.
Methods: The study population consisted of 260 unrelated T1D patients (137 male and 123 female, median age 17.8±4.4). Based on recommended limit lipid levels for T1D, the patients were divided into 2 groups with TC>4.4 mmol/L or TG>1.7 mmol/L and a group with normal TC≤4.4 mmol/L or TG≤1.7 mmol/L. APOE genotyping was performed using TaqMan genotyping assays. Independent t-test was used to compare the continuous parameters and chi-squared test to determine differences in allelic and genotype frequencies among patients.
Results: Patients with elevated TC or TG levels had higher body mass index and poor glycemic control (HbA1c). Additionally patients with elevated TC levels had higher body weight and increased diastolic blood pressure. Most common allele was e3 (84.8%) and most common genotype was e3/e3 (71.5%). Carriers of genotype combination e3/e4 (OR=2.31, 95%CI=1.18-4.49, P=0.012) or E4 allele (OR=2.42, 95%CI=1.25-4.69, P=0.008) have higher TC concentration.
Conclusion: In conclusion, serum lipid levels in T1D patients are significantly influenced by genetic background and not only the environmental factors.

PS18.05

Genome-wide association study of asthma in the Spanish population reveals a new susceptibility locus

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Many genes contributing to asthma heritability remain undiscovered besides the firm susceptibility genes exposed to date. Most genome-wide association studies (GWAS) for the trait have been performed in northern European populations and have analyzed the genetic variation provided by HapMap. As genetic risks varies among populations, here we have performed a three stage GWAS with unrelated Spanish subjects, which harbors 4-20% North African admixture. By leveraging the information provided by The 1000 Genomes Project, we analyzed ~6.5 million common variants (MAF>5%, Rsq>0.3) in 380 asthma cases and 552 population-based controls, using the Axiom Genome-Wide CEU array (Affymetrix). We performed a prioritization of 18 loci associated at suggestive significance (p≤5x10-5) that were followed up in a second sample of 482 cases and 1209 controls and a third sample of 346 cases and 137 controls, genotyped using iPLEX Gold assays (Sequenom). Two loci (2q33.2, 3p14.1) were nominally associated in the second sample, while only 3p14.1 achieved nominal significance in the third sample as well. A fixed effects meta-analysis of the 3,106 individuals confirmed the association of 3p14.1 with asthma (OR=0.49, 95% CI=0.38-0.64, p=1.28x10-7). To our knowledge, this is the first GWAS of asthma in the Spanish population, allowing to identify a new susceptibility locus.
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PM18.06

Glutathione S Transferase M1and T1 gene variants increases risk of asthma in Indian children: A case control study

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Abstract
Background- Asthma is a complex genetic disorder. Glutathione S-transferases (GST) gene plays a major role in the detoxification of metabolites of oxidative stress resulting in inflammation leads to asthma. Therefore, the hypothesis that GSTT1 and GSTM1 gene variants are associated with asthma was examined.
Material and methods- Hospital based case-control study, 150 cases and 150 age and sex matched controls, aged 1 year -15 years were recruited. Cases included were those children presenting symptoms of asthma according to EPR 2007 and excluded were children with other respiratory diseases. Children with no present and past history of asthma were enrolled as controls. Spirometry was done in cases age ≥ 6 years. Binary logistic regression and chi square test are used to find out the association.
Results- Of 150 cases and 150 controls, GSTT1 null allele was found more prevalent in asthmatics (36 %) than in the controls (17.3 %), which yielded a nearly thrice fold risk towards asthma (OR=3.17, 95 %CI =1.86-5.42, p = 0.000). Increased risk of asthma was also found in individuals having GSTM1 null allele in asthma patients (46.6 %) than in controls (39.4 %) (OR=2.25, 95 %CI =1.39-3.63, p = 0.003). Value of FEV1/FVC ratio was significantly lower in asthmatics having GST gene variants (P value< 0.05).
Conclusions- GST gene variants increases asthma risk and also associated with decline lung function growth in Indian children.
Funding- Indian Council of Medical Research, India

PS18.07

The unique evolutionary signature of autism genes

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Autism spectrum disorder (ASD) is a prevalent heritable neurodevelopmental disorder, characterized by social, and communication disabilities that greatly reduce reproductive fitness. The maintenance of such heritable and low-reproductive trait in the human population is an evolutionary enigma that likely left a signature in ASD genes. To test this hypothesis, we studied the allelic characteristics of 441 ASD genes and 15,144 non-ASD genes in a whole-exome sequencing dataset, which included data on 503,481 single-nucleotide variants (SNVs) in 1351 European Americans, and 1088 African Americans individuals. We discovered that ASD genes were significantly longer, and less variable than non-ASD genes (2684 vs. 1632 bp, and nucleotide diversity $\pi = 0.036$ vs. $\pi = 0.046$ respectively; $P<0.001$). The mutational dearth in ASD genes was particularly eminent when considering only SNVs with functional consequences, a possible effect of negative selection. Interestingly, ASD genes also showed signs of positive selection, but these were less evident ($P<0.09$). We used these genomic characteristics to generate a multivariate model that classified ASD from non-ASD genes with 72% accuracy. These finding suggest that ASD genes have a unique evolutionary signature that could be used to identify new candidate genes for this condition.

PM18.08

Mapping shared markers across a polynesian population in the genomic age

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Identifying susceptibility variants for multifactorial human traits can be complicated depending on the understanding of the genetic background on which it arises. This is especially difficult in small, under-studied populations without readily-available control cohorts. In such populations, low-penetrance variants in complex disease can be challenging to confidently identify when normal variation and allele frequencies are uncharacterised. We present an investigation of biliary atresia (BA), a usually sporadic malformation of the biliary tree. Worldwide, BA leads to half of all paediatric liver transplants, and is always fatal without major surgical intervention. The causes of BA remain unknown; autoimmune processes and genetic background may both play a part. In *Māori* and Polynesian populations the incidence is elevated three-fold compared to Europeans. We have identified a large Maori family (iwi) exhibiting an extremely elevated incidence of BA (1:100–300). To circumvent some of the problems in studying complex traits in Maori we

have adopted a non-parametric, family-based approach to localise a presumptive genetic factor conferring this susceptibility. Assuming a single, segregating susceptibility factor contributes to BA in our family cohort, we have used the software Beagle, Germline and in-house methods to examine haplotype sharing between affected pairs across this family. We have constructed maps of long, Identical-By-State (IBS) segments across the collective genomes of affected individuals without reliance on pre-existing assumptions about allele frequencies, a definitive inheritance model or exclusion of unidentified phenocopies. Further work aims to integrate rare variation from next generation sequencing data to further resolve shared haplotypes and define candidate regions in which a susceptibility variant could lie.

PM18.10

Evaluating the association between candidate gene and disease status in genome-wide association studies: An enhanced version of Cochran-Armitage trend test

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Introduction: In genome-wide association (GWA) studies the evaluation of the association between candidate gene and disease status is widely carried out using Cochran-Armitage trend test. However, only a small number of research papers have evaluated the distribution of p-values for the Cochran-Armitage trend test. In this paper we explore the genetic association for case-control design that draw the inference of the equality of the genotype frequencies in case and control and thus introducing an enhanced version of Cochran-Armitage trend test.
Materials and Methods: simulation studies have been applied to demonstrate the validity of the proposed methods for the inference of trend test. In order to study the finite sample properties of them, Monte Carlo experiments are used. The proposed methods are simultaneously based on the same simulated data in order to provide a meaningful comparison of various algorithms. The simulations are done based on the 5000 iterations. In order to make a comparative evaluation of the procedures, we seek the certain desirable features such as the actual significance level.
Results: Both simulation studies and real data studies have been used to validate the theoretical results. The QQplot of the p-value of the proposed tests shows that the p-value using the introduced method fits more with the uniform distribution that admits this method can be nominated to draw the inference.
Conclusions: Based on achieved results it is concluded that the proposed method which needs less assumption in comparison with the conventional method can be successfully used to test the genetic association.

PS18.11

Reconstruction Of The Ancestral Haplotype With c.-23+1G>A Splice Site Mutation Of The Gjb2 Gene In Certain Populations Of Eurasia

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To date, the c.-23+1G>A (IVS1+1G>A) splice site mutation is one of frequent mutations in the GJB2 gene (MIM 121011, 13q11-q12) in patients with congenital deafness in certain populations of Eastern Europe, Caucasus, Middle East, Central Asia, Southern and Eastern Siberia. Previously, it was shown that this mutation is widespread in Yakutia (Eastern Siberia) due to founder effect. For reconstruction of haplotypes with mutation c.-23+1G>A and for comparative analysis with other populations of Eurasia (Mongolia and Turkey) 9 SNPs flanking the GJB2 gene and previously reported in (Tekin et al., 2010) have been used. Total sample was represented by 126 individuals homozygous for the c.-23+1G>A mutation: Yakuts (n=111), Tuvinians (n=6), Mongolians (n=4), Turks (n=3), Evenk (n=1), and Russian (n=1). Five different haplotypes were identified with variety among the Mongolians (4 haplotypes) and Yakuts (3 haplotypes). The reconstruction of haplotypes with a c.-23+1G>A mutation revealed common SNP-haplotype C-A-G-A-C-C-A-T-G, which is present in most of studied chromosomes of patients homozygous for this mutation: 100% for Tuvinians, Turks, Russian, and Evenk, 99% - for Yakuts, and 67% - for Mongolians. The results suggest that the c.-23+1G>A mutation in the GJB2 gene has a common origin in Eurasia.
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PM18.12

Funded access to large prospective cohorts in Europe through the biobanking infrastructure BBMRI-LPC

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Most of the present knowledge on diagnostics, diseases and drugs was obtained by careful investigation of human biospecimens and related medical data. Central for such investigations are biobanks that systematically collect and store biological samples with information of study individuals before and up to the onset of disease and beyond. A critical factor for success is facilitation of structured access to biobanked materials in an ethically- and privacy-compliant manner. In 2013, an EU-funded multinational BBMRI-LPC-project (Biobanking and Biomolecular Resources Research Infrastructure - Large Prospective Cohorts) was established in mission to facilitate 'free-of-charge' access to samples and health data across country borders through open scientific calls. BBMRI-LPC unites prospective study sets from 17 countries with 22 different cohorts involving over 1 million study participants.
The 1st BBMRI-LPC scientific call, released in July 2014, resulted in 9 proposals. Four of these, applying innovative technologies, notably metabolomics, to early discovery of various cancers and cardiovascular diseases, were approved. The proposals involved in total 18 prospective cohorts of which 11 are now active in their access procedures. In order to map the access steps and potential bottlenecks, BBMRI-LPC records the versatile procedures. Following the successful 1st call, BBMRI-LPC launches its 2nd call in March 2015 inviting researchers in EU-member and associated states to submit their cutting edge proposals on common chronic diseases. The poster describes the current records on 1st call access, and presents the specifics of the 2nd call, through which the unique prospective collections in Europe can yet again be harnessed for innovative research initiatives.

PS18.13

Evaluation of CVD genetic risk in the Lithuanian population

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According to Perk J et al., 2012 Lithuania is among the countries of very high cardiovascular disease (CVD) risk. Common CVD risk algorithms are non-universal and either overestimate or underestimate the risk. It is important to evaluate genetic risk factors in order to create an algorithm as a measure of an accurate CVD risk evaluation of local patients.
Lithuanian population is represented as 253 unrelated individuals (127 women and 126 men) recruited from the ethnolinguistic regions - Žemaitija (113) and Aukštaitija (140). DNA samples were extracted from venous blood either by phenol-chloroform or magnetic beads isolation methods. Genotyping was performed using the Illumina HumanOmniExpress-12 v1.1 assay. Sixty CVD-associated SNPs were selected for the analysis. The R v3.0.3 was used for statistical analysis.
The number of risk alleles per person in the population and intra-population groups (men and women, Aukštaičiai and Žemaičiai) was determined. The mean value was 55.81, median 56, mode 59, minimum value was 39 and maximum - 74. No statistically significant differences among intra-population groups were found. All individuals were divided into the groups by the number of risk alleles (39-50 (small); 51-62 (medium); 63-74 (large)). 68% of individuals fell into the medium and 13% into the large group. Risk alleles of SNPs have a small effect size and regarding their additive effect it can be

concluded that majority of the Lithuanian population individuals are at an average CVD genetic risk.

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PM18.14

Fine mapping of the childhood asthma susceptibility 17q21 locus

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Within the GABRIEL consortium we identified 17q21 as the major childhood asthma susceptibility locus. Here, numerous single nucleotide polymorphisms (SNPs) in high linkage disequilibrium spread across seven genes, covering 238 kb.

While the finding was widely replicated, it is yet unclear whether one or more causal SNPs exist in the region and to which genes the signal annotates. Therefore, we fine mapped the locus and evaluated how certain alleles influence disease susceptibility and genes expression.

Data was acquired for 1,454 children (763 asthmatics) from the German MAGIC and ISAAC II studies, using Illumina Chip and MALDI-TOF MS genotyping, direct sequencing, and imputation. Allele-specific *cis*-effects on the 17q21 genes expression were determined in peripheral blood mononuclear cells (PBMC) for selected asthma-associated SNPs in 50 adult non-asthmatics.

The initial genome-wide dataset provided information for only 36 SNPs from the 17q21 locus. We substantially increased the number of genotyped (n=68) and analyzed (n=156, 88 imputed) polymorphisms for their relation with asthma. Eighty-five of them showed disease associations (58 with $p<10^{-5}$). The major signals derive from five distinct tagging bins comprising *GSDMB-GSDMA* region, with rs8079416 being the top hit ($p=6.08*10^{-8}$). Association data was also available for variants of the recently validated *LRRC3C*. Polymorphisms (rs12603332, rs4795405 and rs8079416) located in a cluster, representing three highest association peaks, influence *ORMDL3* and *GSDMA* levels in PBMC [non-stimulated and cells, stimulated with allergens (PHA, Derp1, LpA and Ppg)] in an allele-specific manner.

Multiple 17q21 childhood asthma signals exist. The major peaks originate from the *GSDMB-ORMDL3-LRRC3C-GSDMA* region, suggesting that these genes could functionally underlie those associations.

PS18.15

Development of a comprehensive next-generation sequencing panel for UMOD and association analyses of SNPs and methylation in UMOD, UMODL1, and UMODL1-AS1 genes for kidney disease in multiple populations.

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Chronic kidney disease (CKD) is a major problem worldwide, leading to end-stage renal disease (ESRD), with associated cardiovascular disease and premature mortality. *UMOD* encodes uromodulin (Tamm-Horsfall glycoprotein) which is protective against kidney disease and is associated with Mendelian and multi-factorial CKD.

Using www.ampliseq.com we designed a next-generation-sequencing panel to comprehensively sequence 23,928 bp extending ~2 kb up and downstream of *UMOD* on chromosome 16p12.3. There is 96.4% coverage of the entire region with 100% coverage of exons using 89 amplicons (125-375 bp) sequenced on an Ion Torrent Personal Genome Machine (PGM™) generating >900M aligned bases per 318 chip. Linkage disequilibrium was established for identified SNPs and common tag SNPs genotyped in 2,000 individuals using a case-control approach. A comprehensive literature review was conducted for *UMOD* to facilitate meta-analysis for SNPs that were present in more than three studies with similar kidney phenotypes.

Quantitative methylation data was available for 255 cases with CKD compared to 152 controls without kidney disease from three independent studies. Following stringent quality control, and adjustment for multiple testing, five CpG sites were associated in *UMOD*, with subgroup analysis for diabetic nephropathy revealing cg03140788 as the most significant with P=0.00000000037. We also considered *UMODL1* and overlapping *UMODL1-AS1*, revealing twenty-four associated CpG sites, with $P_{max}=2.9x10^{-32}$ (cg16624482) for non-diabetic ESRD.

We have developed a cost-effective approach for genotyping individuals or larger population-based cohorts using 20 ng of input DNA. Genes were characterised for SNPs and CpG sites. Strong association was observed for CKD, which was supported by independent replication.

PM18.16

Association between three gene polymorphisms and chronic periodontitis among Romanian population

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Introduction: Chronic periodontitis (CP) is a multifactorial disease. The TGFb, IL6 and SELL gene polymorphisms may be involved in the aetiology of CP. The aim of this study was to analyze the association between chronic periodontitis and TGFb C-509T, IL6 G-174C and SELL C605T genetic polymorphisms in the Romanian population.

Materials and Methods: A total of 330 unrelated individuals, with at least 20 teeth in the oral cavity, were enrolled in the study. One hundred sixty five chronic periodontitis patients and 165 healthy controls were selected. The subjects were 44±3.8 (34-60) years old. Control subjects had no evidence of clinical attachment loss and they were age and gender matched with patients. The diagnosis of CP was made on the basis of standardized clinical criteria. The polymorphisms were genotyped using polymerase chain reaction-based methods.

Results: The distribution of the investigated polymorphisms in both groups was in Hardy-Weinberg equilibrium. When the polymorphisms were considered independently, only the TGFb C-509T was associated with CP ($p<0.05$). Additional factors seem to influence this association. The risk for CP was also increases by the association between TGFb and gender (risk was higher in women) or SELL 605T variant (p=0.001) and by the presence of IL6 -174C variant in smokers (p=0.05).

Conclusion: TGFb considered individually, or in association with gender and SELL 605T or IL6 -174C variants in smokers, was associated with CP in the Romanian population.

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PS18.17

Susceptibility to colorectal cancer is influenced by interaction between genetic variants and plasma vitamin D level

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Introduction: Vitamin D deficiency is associated with risk of colorectal cancer (CRC), however causality remains questionable. We investigated the possible role of gene-environment (GxE) interactions between genetic variants and plasma vitamin D levels on colorectal cancer risk.

Material and Methods: 3114 CRC cases and 2939 population based controls were collected as a part of Study of Colorectal Cancer in Scotland (SOCCS). Plasma was assayed for 25-OHD by liquid chromatography-tandem mass spectrometry. All sample were genotyped using Illumina HumanHap 240K/300K array (675 cases and 896 controls), Omni5M (131 cases), OmniExomeExpress array (1379 cases) or custom 50K Illumina Array (929 cases and 2043 controls). Logistic regression models with genotype-vitamin D interaction terms were adjusted for sampling month, age and gender. 115 normal colorectal mucosa samples with available Illumina HT12 expression

array were used to explore co-expression of vitamin D receptor (VDR) gene and the genes identified through GxE analysis.
Results: Initial scan for GxE interaction in the subset of 24,471 variants overlapping between different platforms identified variant at 19q13.41 reaching significance level after correction for multiple testing. When we constrained analysis to the subset of genes correlated with VDR expression level in normal mucosa ($p<0.05$, n=2932), an increase in genomic inflation factor ($\lambda=1.10$ vs $\lambda=1.02$ for overall analysis) was observed, suggesting enrichment for GxE interactions.
Conclusion: Preliminary GxE analysis identified a new candidate locus associated with the CRC risk. We observed enrichment for GxE interaction within genes correlated with VDR expression, suggesting a causal relationship between vitamin D and risk.
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PM18.18
Genetic trace of postglacial and Neolithic movements in Southeast Asia

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Island Southeast Asia (ISEA) is still one of the less genetically characterized region in the world, and it is often absent from ancestry studies of the Pacific even though it is a key location for understanding the population history of that region. Recent genetic studies suggest that the simplistic and most widely accepted model of an Australo-Melanesian first settlement ~60 ka followed by an Austronesian expansion around 4.5 ka does not fully capture the complexity of ISEA demographic history. To clarify the main dispersal routes and their impact in ISEA population prehistory, we performed a comprehensive study with a total of 114 newly complete mtDNA genomes affiliated in haplogroups previously associated with several demographic events in SEA. The most-parsimonious phylogenetic trees were reconstructed including all published data. The statistical analyses included ρ statistics and maximum likelihood (ML) to estimate the coalescence times of clades, and Bayesian methods to evaluate changes in effective population size through time. Our results show two main demographic events contributing to the gene pool of ISEA populations. One was the result of climatic changes and subsequent landscape alterations which lead to the postglacial dispersal of mtDNA lineages N9a6a, R9ab1a1a, B4c1b2a2, B5b1c, F3b1 and possibly R9c1a from both Mainland and ISEA, representing the major signal in ISEA. However dispersal of Austronesian-speaking Neolithic populations from Taiwan towards insular SEA were detected introducing mtDNA lineages D5b1c1, B4b1a2, F1a4a and Y2a1, that together with previously analysed M7c3c, correspond to ~20% of ISEA lineages.
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PS18.19
Candidate gene association study of chronic obstructive pulmonary disease using targeted high throughput sequencing

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Chronic obstructive pulmonary disease (COPD) is a relatively common lung disease in Sweden with severe impact on health and quality of life. Smoking is an important risk factor. Not all smokers develop COPD and non-smokers can be in elevated risk for COPD, suggesting genetic factors modulating lifetime risk. Today, little is known about the impact of genetic variation on COPD susceptibility, progress and severity.
Our aim is to investigate candidate genes, with emphasis on those important for lung development, for single nucleotide variants (SNVs) predisposing to COPD.
We captured and sequenced 22 genes implicated in lung development and 71 genes and regions previously associated with COPD. Subjects with COPD and controls without COPD were retrieved from the Swedish Obstructive Lung Disease in Norrbotten (OLIN) sample. Both groups were matched for age, gender, weight and height, and contain the same number of smokers.
We found 75 SNVs in significant association with COPD and all showed a strong effect size as either low or high odds ratios (0.2-8.1). These variants are mainly in genes that cluster in pathways associated with cell proliferation, which includes genes in branching morphogenesis of the lung pathways involved in lung development and damage repair.
Our results confirm previous findings but with higher effect sizes of associated variants compared to similar studies, most likely attributable to the genetic background in our study population. These findings support the idea that genetic variants affecting lung developmental genes are important determinants of adult lung function.

PM18.20
Common CNVs in a population-based cohort reveal several associations in the transcriptome and consecutive changes in the metabolome

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Copy-number variations (CNVs) are widespread structural changes in the genome having a global impact on the transcriptome, and also recognized risk factors for complex diseases. However, the functional contribution of CNVs to human health is still not well understood. The variability of gene expression is not explained by genetic diversity alone, therefore integration of systems-level information, omics data, will provide a better understanding of human biology and disease etiology.
To assess cis-effects of common CNVs on gene expression and on complex phenotypes in the Finnish population-based cohort DILGOM (N=510), we performed association analyses of CNVs to both the peripheral blood leucocyte transcriptome and nuclear magnetic resonance (NMR) spectroscopy -measured serum metabolome . We used linear regression to estimate the associations between 685 CNVs and expression levels of 37, 022 transcripts. A similar model was used for the serum metabolome, consisting of 31 lipid and lipoprotein-related metabolite measures.
After correction for multiple testing, 38 CNVs showed associations ($p<2.28e-06$) with gene expression levels and 14 of these CNVs showed further association ($p<0.05$) with metabolite levels. As an interesting example, a CNV in region 17q12 associated with SLFN13 and the ratio between apolipoproteins apoB and apoA1 in women ($p=2.12e-04$). Furthermore, the DILGOM sample seven-year-follow-up of transcriptomic and metabolomic profiles will soon allow us to perform an intra-cohort replication.
Our results suggest that integration of omics data is a powerful approach to reveal novel connections between phenotypic variation and genomic diversity.

PS18.21
Genomic diversity and distribution of CNVs in Lithuanian population

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Although copy number variation (CNV) has received much attention, the knowledge about CNV's characteristics as occurrence rate, distribution in genome among populations and within the same population is still insufficient. In this study, we used *Illumina 770K HumanOmniExpress-12v1.0* (and *v1.1*) arrays to examine diversity and distribution of CNVs in 286 healthy individuals of two main ethnolinguistic groups of Lithuanian population (Aukštaičiai, Žemaičiai) . For primary data analysis we employed *Illumina GenomeStudio™ Genotyping Module v1.9* and two algorithms, *cnvPartition 3.2.0* and *QauntiSNP 2.0* for high-confidence CNVs identification. In total, 478 autosomal CNVs were detected by both algorithms and those were clustered in 87 copy number variation regions (CNVRs), spanning ~12.5 Mb of genome (Table 1). At least 8.6% CNVRs were unique and had not been reported in the *Database of Genomic Variants*. Most of CNVRs (57.5%) were rare with frequency ≤1%, whereas common CNVRs with at least 5% frequency comprised only 1.1% of all CNVRs. About 30% of CNVRs were shared among Aukštaičiai and Žemaičiai and the remaining CNVRs were specific to each group. Many of the detected CNVs (66.3%) overlapped with known *UCSC* gene regions. CNV distribution analysis between ethnolinguistic groups showed the differences in CNV occurrence and frequency both in the indi-

vidual level and at the population level, however there were no statistically significant differences found (p-value 0.9585, α= 0.05).
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Table 1. Lithuanian population characteristics of CNVs and CNVRs.

CNVs	**Aukštaičiai**	**Žemaičiai**	**Overall**
Sample size	166	120	286
CNV carriers (%)	103 (62%)	84 (70%)	187 (65.4%)
Number of CNVs	262	216	478
CNVs per person	1.58	1.8	1.67
Duplications	123 (47%)	103 (47.7%)	226 (47.3%)
Deletions	139 (53%)	113 (52.3%)	252 (52.7%)
Mean size of CNVs	133 kb	152.8 kb	141.9 kb
Median size of CNVs	70.7 kb	86.2 kb	78.2 kb
CNVRs			
Total number of CNVRs	49	38	87
Mean size of CNVRs	138.4 kb	144.1 kb	143.7 kb
Median size of CNVRs	73.6 kb	85 kb	86.8 kb
Genome coverage by CNVRs	6.8 Mb	5.5 Mb	12.5 Mb

PM18.22

Analysis of genomic regions associated with Coronary Artery Disease reveals continental-specific risk SNPs in North African populations.

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Coronary artery disease (CAD) is a complex disease and the leading cause of death in the world. Populations of different ancestry do not always share the same risk markers. In recent years, several genomic regions have been robustly associated with CAD in different genome-wide association studies mainly conducted on people of European descent. These kinds of data are lacking in African populations even though heart diseases are currently an important cause of premature death and disability.
In this study, 384 single nucleotide polymorphisms (SNP) located in four genomic regions associated with CAD (1p13, 1q41, 9p21 and 10q11) were analysed in a novel set of 274 case-control samples from Morocco and Tunisia with the aim of analyse for the first time if the associations found in European populations could be transferable also to North Africa.
The results indicated that the variation in these four genetic regions had an important role for CAD predisposition also in North Africa. However, the individual SNPs associated with CAD in Africa were different from those identified in Europe in most cases (1p13, 1q41, and 9p21). In addition, significant differences in the haplotype blocks and in the linkage disequilibrium patterns among North Africa and Europe were detected.
The disparity in markers associated to CAD susceptibility between North Africa and Europe is probably due to the significant population differences found in the chromosomal architecture of these 4 CAD risk regions.
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PS18.23

Exome-sequencing in a large family-based and population-based study identifies a large-effect missense variant associated with depression

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Depression is a prevalent psychiatric disorder with a strong genetic component (40-50%). Efforts to uncover common genetic variation underlying depression have so far failed. Here we explored whether relatively large-effect-rare variants influence the risk of depression. To investigate high-risk genetic variants associated with depression, we adopted a step-wise approach. Within the Erasmus Rucphen Family (ERF) study, we identified families and individuals informative for the segregation of large-effect-rare variants using linkage and haplotype analyses with a sparse genome-wide genotyping array. We then used exome-sequence data of these individuals and the filtering approach to identify potentially high-risk variants. Next we associated the identified variants with depressive symptoms
in the ERF study (N=1,327). Finally, we replicated the findings in the population-based Rotterdam study (RS, N=2,356). We identified a missense C>T variant on chromosome 9p24 shared by affected haplotype carriers in the family. The variant was conserved and characterized as a damaging variant. The frequency of this variant was increased ~10 times (1.4%) in ERF compared to the general population (1000Genomes=0.16%). Carriers (N=51) were distributed across 5 generations. Significant association was observed with depressive symptoms (p-value=$9*10^{-04}$) giving an increase of depressive symptoms by 2.47 per-allele. The variant explained ~5% of the genetic variance of depressive symptoms in ERF. Significant association (p-value=0.032) and a large effect (effect=3.60) was observed on depressive symptoms in RS (N(carriers)=23). The gene harboring the C>T variant is a novel gene for depression. Functional studies are required to ascertain the mechanisms through which the variant influences the risk for depression.
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PM18.24

Association analysis of thirty DNA sequence polymorphisms with diabetic retinopathy in Pakistan

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Introduction: Polymorphic single nucleotide DNA sequence variations in components of different regulatory and metabolic pathways have been reported to be associated with diabetes and its related complications worldwide. In the present study we assessed the role of 30 such DNA polymorphisms in the onset of diabetes-induced retinopathy (DR) development in Pakistani type 2 diabetic subjects.
Materials and Methods: A total of 570 age and gender-matched individuals that included diabetic retinopathy (177=99 non-proliferative DR+78 proliferative DR), diabetic non-retinopathy (DNR; 193) and controls (200) were genetically screened for polymorphisms in *ACE*, *PAI-1*, *SDH*, *eNOS*, *PON1*, *GSTT1*, *GSTM1*, *GSTP*, *VEGF*, *RAGE*, *TNF-α*, *ATF6*, *HSP70*, *MTHFR* and *MMP9*. The genotyping techniques included allele specific PCR, RFLP and Sanger sequencing. Statistical comparisons were carried out by gender and subclinical class adjusted logistic regression analysis.
Results: Out of thirty SNPs studied, significant associations (p<0.05) were observed for *HSP70* with diabetes, DNR, DR and non-proliferative DR; *MTHFR* (rs1801133) with DNR; *SDH* (rs2055858) with DR and proliferative DR, *SDH* rs3759890 and rs2055858 were also found to have synergistic role in disease development; *VEGF* (rs1570360) with proliferative DR; *TNF-α* and *GSTT1M0* with proliferative and non-proliferative DR; *ACE* with non-proliferative DR.
Conclusions: This study of genetics of diabetic retinopathy in Pakistan revealed that there is significant associations with the disease of 7 polymorphisms, which are components of folate and homocysteine metabolism, angiogensis, polyol pathway, renin-angiotensin system, antioxidant pathway and heat shock protein, with different studied sub-classes of Pakistani type 2 diabetic subjects.

PS18.25

A case-control association study of diabetic retinopathy in Edinburgh Type 2 Diabetes Study

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Background: Type 2 diabetes (T2D) is a serious public health concern resulting in a number of macrovascular and microvascular complications. Diabetic retinopathy (DR) is an important microvascular complication and a leading cause of blindness worldwide. DR is a complex trait, dependant on number of factors (e.g. glycaemic exposure, blood pressure), and increasing evidence suggests that genetic variation may also influence the susceptibility to DR. Previously published genome-wide association studies (GWAS) have identified loci underlying DR within the regions of 1q13-42, 2q31-47, 6q22-27 and 13q14-32. However, these loci have not been replicated yet and studies used different grading schemes which confound interpretation of results. We aimed to explore the data from the Edinburgh Type 2 Diabetes Study (ET2DS) to identify potential association of genetic variants with DR in T2D patients.
Methods: A case-control association study was performed in 299 diabetic cases with DR and 640 diabetic controls. Cases were characterised according to Early Treatment Diabetic Retinopathy Study (ETDRS) classification. Genotype data obtained by the Illumina MetaboChip® array was used for association analysis using PLINK. We used logistic regression analysis adjusted for sex, duration of diabetes, systolic and diastolic blood pressure and glycosylated haemoglobin.
Results: Despite being one the largest study of its kind to date in Caucasians, no significant associations between genetic variants and DR were found.
Conclusions: Further studies with an increased sample size are needed to identify loci associated with DR. The identification of new loci will reveal a better understanding of the aetiology of DR and could help to identify environmental factors contributing to the disease development.

PM18.26

Supporting human genetics research for 30 years, and into the future

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Public Health England's secure site at Porton Down this year celebrates 30 years of supporting human genetic research. In this time the European Collection of Cell Cultures (ECACC), has processed human blood samples, from tens of thousands of individuals and their family members, cryopreserving, storing and distributing, viable primary lymphocytes, cell lines and nucleic acids, to scientific researchers involved in the study of hundreds of genetic diseases. Originally established to support the Human Genome Mapping Project (HGMP) the service has evolved, and continues to enable the MRC DNA banking Network, the Wellcome Trust and dozens of industrial and academic groups and charity funded projects to achieve their goals. The service accurately processes thousands of samples in a high throughput manner with quality assurance supported by barcode tracking, testing of samples for sterility, and STR profiling to ensure that genetic identity is maintained from blood through to cell line and extracted nucleic acids. Cells are optimally cryopreserved and stored, in liquid nitrogen tanks in a dedicated warehouse, where samples can be confidently retrieved, even decades after storage. We show how our data management, human tissue authority (HTA), quality compliance systems, and infrastructure have adapted and developed over the last 30 years. The significant challenges of maintaining and developing this high quality system, in a not-for-profit organisation, adapting this infrastructure to help serve the emerging field of personalised medicine, and the development of large cohort induced pluripotent stem (iPS) cell banks are explained.

PS18.27

Epidemiology of Elis van Creveld syndrome in Europe: a registry based study

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Ellis-van Creveld syndrome or chondroectodermal dysplasia (EVC, MIM 225500) is a rare autosomal recessive skeletal dysplasia characterized by short limbs and ribs, postaxial polydactyly, dysplastic nails and teeth and congenital heart defects. We present the largest population-based epidemiological study to date using data provided by the European Surveillance of Congenital Anomalies (EUROCAT) network of congenital anomaly registries. The study population consisted of 42 cases of EVC identified between January 1990 and December 2012 in 34 European registries. The mean prevalence of EVC was 0.53 per 100 000 births. There were 25 (59.5%) terminations of pregnancy after prenatal diagnosis, 2 (4.7%) fetal deaths, and 15 (35.7%) live births. The most common anomaly was polydactyly (27; 87.1%). Congenital heart anomalies, mostly septal defects, were present in 20 (64.5%) cases. Most cases (85.7%) are suspected prenatally at 18.8±4.2 (range 12-33) gestational weeks. The ultrasound examination usually reveals shortening of long bones, narrow thorax, hexadactyly and cardiac defects. Pregnancies are mainly (25/30, 83.3%) terminated at 20.1±4.6 gestational weeks, reducing the number of live births to one third of the total prevalence rate (0.19 per 100 000 births, or 1 in 528 044). In conclusion, EVC is a very rare disease affecting 1 in 188587 births. Early diagnosis is important for timely counselling of couples and multidisciplinary management of affected children. The study is part of the EUROCAT Joint Action funded by the EC, under the framework of EU Health Programme 2008-2013, Grant Agreement 20102204 (Executive Agency for Health & Consumers).

PM18.28

Genetic and Epigenetic Analysis of ELMO1 for Association with Kidney Disease

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End stage kidney disease (ESKD) is the most severe stage of chronic kidney disease (CKD). In 2011, the incidence of renal replacement therapy for ESKD in Europe was 117 per million population, creating substantial healthcare costs. Selected SNPs and differential methylation, with corresponding changes to gene expression of ELMO1, have been associated with CKD leading to dysregulation of extracellular matrix proteins. We sought to evaluate SNPs and CpG sites in ELMO1, performing a meta-analysis of independent studies and exploring relevant methylation quantitative trait loci (meQTLs).

A comprehensive literature review conducted for ELMO1 facilitated meta-analysis for SNPs that were present in greater than three studies with similar kidney phenotypes. The Infinium® methylation 450K BeadChip array (Illumina, Inc, USA) was used to analyse DNA methylation across the methylome in 255 CKD cases and 152 controls without kidney disease. Following stringent quality control, significant CpG sites in ELMO1 were identified. The top ranked CpG site for this gene was cg05642546 (P=4.8x10-27). Genome-wide SNP data (n=561,233 SNPs; 372 individuals) from the Illumina 660K array was analysed in PLINK with methylation data from CpG sites in the ELMO1 gene to identify meQTLs associated with ESKD and healthy individuals. No genome-wide significant cis-meQTLs were identified for ELMO1, however genome-wide significant results for trans-meQTLs appear to affect ELMO1 through regulatory effects.
We have provided a genomic map of ELMO1 that combines published and novel data to help resolve conflicting association data and suggest a mechanism of how ELMO1 influences kidney disease.

PS18.29

Exome analysis of rare and common variants within the NOD receptor pathway

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Background: Pediatric inflammatory bowel disease (PIBD) is a chronic gastrointestinal autoimmune disorder in which genetic predisposition plays a more substantial role. Rare and private mutations may have a stronger impact in the susceptibility of PIBD compared to common variants. This study looks at the burden of any coding mutation in PIBD patients within genes of the NOD2 pathway using data derived from exome sequencing.
Methods: Cases and controls were recruited through tertiary referral clinics at University Hospital Southampton. For all exomed patients, genomic DNA was extracted using the salting out method and the samples were exome sequenced at the Wellcome Trust Centre in Oxford. Data were aligned and tested for quality control by using the in-house pipeline and customised scripts. Association testing was conducted between disease status and population control using the SKAT-O test.
Results: 10 genes within the NOD2 pathway have been previously implicated in IBD by GWAS. We observed mutations in 39 of 40 genes comprising this pathway. Despite the small sample size (143 cases and 88 controls), SKAT-O test identified four genes associated with disease status ($p<0.05$). The evidence contributing to the association signal for these genes is primarily driven by rare variants that would not have been assessed in GWAS.
Conclusion: Within our small cohort SKAT-O showed a significant excess of burden of mutation in 4 genes: association was significant for two known IBD genes (NOD2 and IL6) and for two previously unreported genes (BIRC2 and BIRC3). Burden of mutations suggests the possible implication of BIRC2 and BIRC3 in PIBD.

PM18.30

Familial aggregation of late-onset FAP ATTR V30M: looking into asymptomatic carriers

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Introduction: Familial Amyloid Polyneuropathy (FAP ATTRV30M) is an AD systemic amyloidosis, due to a point mutation in the transthyretin (TTR) gene. A wider age-at-onset (AO) variability was uncovered although in Portugal the disease has been characterized by its early onset (<40yrs). However, more and more late-onset (≥50yrs) cases are being ascertained, often matched with older asymptomatic parents. Our aim now was to look into aged-asymptomatic carriers group in order to unravel familial aggregation of late-onset.
Methods: From the largest registry worldwide we analyzed a group of 222 asymptomatic carriers on regular follow-up, aged ≥40 at last observation and their first-degree relatives, belonging to 122 families, using descriptive analysis and the Student's t-test.
Results: Age-at-last-observation varies between 40 and 49 for 103 subjects and was ≥ 50 for 119 of them. Mean age-at-last-observation was 54.06 (SD: 12.2; range: 40-89) and no gender differences were found. We were able to identify 92 transmitting-parents (59 fathers, 33 mothers) with AO. Their mean AO was 56.91 (SD: 12.8; range: 25-80) and no differences in AO were

found between parent's gender. Also, we found a mean AO close to 40 years for siblings of these asymptomatic carriers (mean: 39.91; SD: 8.89; range: 24-65).
Discussion: We confirmed familial aggregation of late-onset cases. We also found that for late-onset cases no gender differences are observed. Due to different clinical aspects of FAP in late-onset patients it is crucial to explore mechanisms that can be related with aging and protective factors that can lead to new therapeutic strategies.

PS18.31
Late-onset cases of FAP ATTR V30M may provide insights on genetic modifiers of age-at-onset (AO)

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Introduction: Familial amyloid polyneuropathy (FAPATTRV30M) is an autosomal dominant systemic amyloidosis, due to a point mutation in the transthyretin (TTR) gene (chr18q12.1). Our registry in Porto (Portugal) has data on 2754 patients (678 families). Although mean AO is 35.3, an important group of late-onset cases (AO≥50) has been diagnosed in more recent years.
Our aim now was to study this group and its differences with early-onset cases.
Methods: Our analysis of 326 late-onset cases (133 families) comprised gender and also their transmitting parent. Descriptive analysis and Student's t-test were used.
Results: Age-at-onset was 60.03 for men and 59.25 for women (NS), as opposed to the general sample where women had a later onset (37.6) than men (33.4). Familial aggregation of late-onset cases is apparent, with some families having up to 11 late-onset cases. Out of 678 probands, ~40% had no affected parent at time of diagnosis, this figure being 86% (115/133) among late-onset probands. These parents had died with no signs of the disease mostly at old-age. No one had an affected parent with early-onset of the disease.
Discussion: While most of FAP probands had one affected parent (as expected in an AD disease), a significant number has a late-onset and no affected parent at time of diagnosis. The study of these families which are protected from the devastating effects of early-onset may provide important clues to disease mechanism and possible genetic modifiers.

PM18.32
Can up-regulation of ERK1/2 and MEK1/2 genes be associated with age-at-onset variability in familial amyloid polyneuropathy (FAP) ATTRV30M?

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Familial amyloid polyneuropathy (FAP ATTRV30M) is an autosomal dominant systemic amyloidosis, due to a point mutation in the transthyretin (TTR) gene. Among Portuguese families, FAP shows a wide variation in age-at-onset (AO) [19-82 yrs] and is characterized by extracellular amyloid deposits of fibrillary TTR and degeneration of peripheral nerves. Extracellular signal-regulated kinases 1/2 (ERK1/2) increase activation in salivary glands and nerves. ERK1/2 kinases (MEK1/2) activation was also up-regulated in peripheral nerves, with phosphorylation of ERK1/2. Therefore, this may represent an early signalling cascade leading to cytotoxic effects of TTR aggregates, resulting in earlier-onset.
Our aim was to study four candidate-genes in this pathway as genetic modifiers of AO in Portuguese FAP ATTRV30M families.
We collected a sample of 106 FAP families with 316 patients. We selected 37 tagging SNPs, genotyped by SNaPshot, sequencing and RFLP. Results were analyzed with the GeneMapper™ v.4.0 software.
We found four significant SNPs in MEK1 gene: rs8039880 (CC), rs11630608 (CC and CT) and rs745796 (GG), genotypes associated with earlier-onset; whereas the GA genotype of rs16949939 was significantly associated with later-onset. For MEK2 gene, only the TT genotype of rs1823059 was significantly related with later AO. For ERK1/2 gene, we did not find significant results.
This study reinforced the role of MEK1/2 genes in FAP signalling mechanism and AO variability. These findings may have important implications in genetic counselling and therapeutic strategies.

PS18.33
The frequency of the MEFV gene mutations and MEFV SNPs haplotypes determined with next generation sequencing technique in FMF patients

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Familial Mediterranean Fever is an autosomal recessive disorder characterized by recurrent attacks of fever, abdominal pain, arthralgias, arthritis and development of amyloidosis over time. In our study, 735 patients who were send to Medical Genetics Department of Kocaeli University, between September 2012-June 2014 for routine diagnosis taken part. We aimed to determine the mutation and SNPs haplotype frequency of the patients MEFV gene who were scanned with the Next Generation DNA Sequencing technique and relationship between diagnostic criteria and detected mutations.
Among 735 patients, mutations were detected in 325 (%44), no mutations were detected in 410 (%56). M694V, E148Q, M680I G/C, V726A, K695R, R761H mutations with the ratio of %41, %17, %13, %8, %2, %2 were detected frequently in our patients respectively. The most frequent symptoms were; abdominal pain, arthritis, fever, chest pain, oral aphthae and erythema were detected with the ratio of %76, %72, %53, %40, %34, %23 respectively among MEFV mutated patients. R202Q alterations were detected in 307 (%42) patients.
Also we analysed MEFV SNPs haplotype among mutated patients. We detected 5 SNPs haplotype group among D102D, G138G, A165A and R202Q polymorphisms and 3 SNPs haplotype group among R314R, E474E, Q476Q, D510D polymorphisms.
Diagnostic power is thought to be increased with the scanning of whole gene with detection of rare mutations and polymorphisms together in FMF disease.
Using this technique in large number of patients are considered to be more meaningful in mutations frequency of the Turkish society studies and constitute a very important place in literature.

PM18.34
Consanguinity and founder effect for Gaucher disease mutation G377S in a population from Tabuleiro do Norte, Northeastern Brazil

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ABSTRACT

INTRODUCTION: Gaucher's Disease (GD) is caused by a β-glucocerebrosidase (GCase) deficiency leading to the accumulation of glucocerebroside in the reticuloendothelial system. The prevalence of GD in Tabuleiro do Norte (TN) (1:4,000) is the highest in Brazil. The purpose of this study was to present evidence of consanguinity and founder effect for the G377S mutation (c.1246G>A) among GD patients in TN based on enzyme, molecular and genealogical studies. **METHODS**: Between March 2009 and December 2010, 131 subjects at risk for GD (GC in dried blood ≤2.19 nmol/h/mL) and 5 confirmed GD patients from the same community were submitted to molecular analysis to characterize the genetic profile of the population. **RESULTS:** Based on the enzymatic and molecular analysis, the subjects were classified into 3 categories: affected (n=5), carrier (n=20), and non-carrier (n=111). All carriers were (G377S/wt). Affected subjects were homozygous (G377S/G377S).
CONCLUSION: The identification of a single type of mutation in carriers and homozygotes from different generations, the history of the community and the genealogy study suggest that the high prevalence of GD in this population may be due to a combination of consanguinity and founder effect for the G377S mutation.

Table 1: Affected subjects, carriers and non-carriers of mutations for Gaucher's disease according to gender, age and enzyme activity (GCase activity in leukocytes and chitotriosidase activity in plasma).

Variables	Subjects according to genetic status			Total	*p*-value
	Non-carrier	Carrier G377S/wt	Affected G377S/G377S		
n (%)	111 (81.6)	20 (14.7)	5 (3.7)	136 (100)	-
Gender Female n(%) Male n(%)	69 (62.2) 42 (37.8)	14 (70.0) 6 (30.0)	3 (60.0) 2 (40.0)	86(63) 50(37)	0.791

Age (years) Average Standard deviation Minimum-Maximum Confidence Interval (95%)	35.51 (20.20) [2-76] (31.71-39.31)	26.70 (14.25) [6-48] (20.03-33.37)	33.40 (10.35) [22-45] (20.54-46.26)	34.14 (19.34) [2-76] (30.86-37.42)	0.242
GCase (nmol/h/mg protein) Average Standard deviation Minimum-Maximum Confidence Interval (95%)	10.47 (3.01) [4.00-20.00] (9.90-11.40)	6.70 (2.03) [2.60-10.00] (5.74-7.65)	1.92 (1.78) [0.13-4.80] (0.00-4.14)	9.48 (3.48) [0.13-20.00] (9.01-10.20)	<0.001
Chitotriosidase (nmol/h/mL) Average Standard deviation Minimum-Maximum Confidence Interval (95%)	51.46 (57.42) [0.80-381.00] (40.66-62.26)	51.54 (32.64) [0.80-144.00] (36.26-66.81)	18,129.40 (8,714.61) [9,110-31,543] (7,308-28,950)	716.10 3,729.00 [0.8-31,543] (83.56-1,349)	<0.001
Type of mutation	wt/wt	G377S/wt	G377S/ G377S	-	-

Source: The authors, 2010.
Comparisons involving three categories simultaneously were done with the Kruskal Wallis test. Pairwise comparisons of categories were done with the Dwass-Steel-Critchlow-Fligner test for non-parametric data.

PS18.35
Incidence of fragile X syndrome in Ireland - an all-Ireland study

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AIMS:
To determine the observed incidence of Fragile X Syndrome (FXS) in the Republic of Ireland (ROI) and Northern Ireland (NI) separately and combined. To compare these observed incidences to estimated worldwide incidences of FXS.
METHOD:
A retrospective clinical and lab database review of positive FXS cases, born between years 2000-2009 inclusive, in both ROI and in NI.
Inclusion criteria: i) Birth place: ROI/NI, ii) Birth year: 2000-2009, iii) FXS confirmed on clinical examination, iv) Full mutation allele (>200 CGG repeats), v) molecular test performed 2000-2014.
RESULTS:
The observed incidence of FXS in males and females, in both the ROI and NI, is ~1:11,000 and ~1:40,000-50,000 respectively. The analysis rate of FXS in ROI and NI is 0.8% and 1.3% of live births respectively however the male: female analysis rate ratio in both ROI and NI was determined to be ~4: 1.
CONCLUSIONS:
The all-Ireland study does not support the commonly held view that FXS is the most common single gene cause of inherited intellectual disability. This first report of the observed incidence of FXS in ROI/NI is approximately 2.5 - 3 times less than the estimated worldwide incidence in males and less for females. The low incidence in males is not due to under-testing as the analysis rates are comparable to international standards. The very low incidence in females is probably exaggerated by lower than expected female analysis rates in both ROI and NI which indicates a need for sex specific criteria to guide clinicians when testing for FXS.

PM18.36
Using Ion Torrent Next Generation Sequencing to identify mutations and alleles spectrum in Glucose 6-phosphate dehydrogenase (G6PD) deficiency among Saudi neonates

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Glucose 6-phosphate dehydrogenase (G6PD) is the most common inherited human enzyme deficiency and estimated to affect more than 400 million people worldwide. G6PD deficiency is an X-linked recessive hereditary condition that causes destruction of red blood cells. Males are more affected than females and the condition is common in African, Mediterranean, and Asiatic populations (i.e. malaria-endemic regions). In the Middle East, the prevalence estimate of G6PD deficiency is the second highest in the world at 6%, the first being Sub Saharan Africa. Saudi Arabia is one of the most affected countries where G6PD deficiency is considered an endemic and all previous studies have been conducted using biochemical analysis. Such analysis limited to disease carries without measuring the severity of the G6PD deficiency of identifying the patient genetic makeup. To date, there are no molecular analysis studies of G6PD gene in Saudi Arabia. Using Ion Torrent next generation sequencing technology; we identify several novel mutations, SNPs and indels and there frequency among 120 neonates patients representing from different population across Saudi Arabia. In this study, we correlated neonate's clinical presentation with their biochemical enzymatic finding and their mutations spectrum finding within G6PD gene. The resultant conclusion can be employed for designing a pre-marital test to reduce the incidence of the disease in our population.

PM18.38
Regional analysis provides insight into the genetic architecture of general cognitive ability

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General cognitive ability is substantially heritable and is predictive of both social and health outcomes. Molecular genetic studies estimate that common SNPs tag around 30% of the phenotypic variation. Here, using 594,756 single nucleotide polymorphisms (SNPs) genotyped in a sample of 6,610 unrelated individuals, the genome wide heritability estimate was decomposed into biologically informative groups with the aim of showing where in the genome the greatest proportion of the polygenic signal lies. The results indicate that the amount of phenotypic variance captured by a chromosome is proportional to its length and that SNPs within genes explain a greater proportion of phenotypic variance than SNPs found outside of genes. A chromosome by chromosome analysis is also provided and for the first time the contribution of common SNPs found on the X chromosome is quantified. Together these results provide further evidence that general cognitive ability is a highly polygenic trait with significant contributions arising in both genic and intergenic regions but with a greater proportion of variation being tagged by SNPs in genes. This unequal distribution of the polygenic signal justifies the use of gene-set analysis methodology to genome wide association study data sets as well as indicates that methods aimed at exploring regions between genes can also identify regions harbouring variants making significant contributions to general cognitive ability.
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PS18.39
ACTN3 R577X and *ACE* I/D gene variants influence specific performance phenotypes in elite sprinters: A study involved ten cohorts of Caucasian and African athletes

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To date, case:control studies investigating the association between the *ACTN3* R577X and the *ACE* I/D gene variants and elite sprint/power performance have been limited by small sample size, the inclusion of sprint and power athletes from mixed sport disciplines, and lack of quantitative measure of performance.
Using a new quantitative approach, we examined the association between the *ACTN3* R577X and the *ACE* ID variants with a total of 527 best personal 100-, 200-, and 400-m times of 349 elite pure sprinters from Australia, Brazil, Greece, Jamaica, Italy, Poland, Russia, Lithuania, Spain, and US. Genotyping of the sprinters was performed using various methods of PCR.
On average, male Caucasian sprinters with the *ACTN3* 577RR or the *ACE* DD genotype had faster best 200-m sprint time than their 577XX (21.19±0.53 vs. 21.86±0.54 sec, p=0.016) and *ACE* II (21.33±0.56 vs. 21.93±0.67 sec, p=0.004) counterparts, respectively. A trend towards a faster best 200-m was noted in the 577RR African sprinters compared to their 577RX counterparts (20.44±0.63 vs. 21.05±0.73, p=0.07). Furthermore, both *ACTN3* 577RR and *ACE* DD sprinters' best personal times were closer to the world record than their 577XX and ACE II counterparts.In conclusion, the *ACTN3* R577X polymorphism individually and in combination with the *ACE* ID polymorphism influence 200- m performance time of elite sprinters, and it is unlikely that athletes with either *ACTN3* 577XX or *ACE* II genotypes will achieve the speeds required for victory in Olympic sprint events.

PM18.40
Analysis of homozygous and compound heterozygous loss-of-function mutations in Finnish exomes in relevance to cardiovascular disorders

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Sequencing Initiative Suomi (SISu) Project recently published the frequencies of loss of function variants (LoF) in 3,000 Finns and showed that the average Finn has more rare loss-of-function variants than other Europeans. To better understand the role of homozygous or compound heterozygous LoF in apparently healthy individuals and study the medial impact and expression profile of the variants, we have studied the +500 -subsample of the FINRISK/DILGOM -study. The baseline of the DILGOM-study was performed in 2007 and all the individuals were re-invited for a follow-up study in 2014.
152 exomes were analyzed with the Illumina GAIIx platform and an Agilent SureSelect All Exon kit. Variants were called together with the SISU project and annotated using Essembl Variant Effect Predictor. We identified a total of 6978 LoF variants in the current sample. Nonsense SNPs were the most frequent variant category (n=3997). Splice site variants (n=2115), initiator codon variants (n= 483), and variants affecting the stop codon (n=383) were also observed. Homozygous or compound heterozygous LoF variants were detected in different 509 transcripts in 259 different autosomal genes.
The variants were reviewed using various databases e.g., Ensembl, OMIM, GWAS catalog, Genecard, NCBI, the 1000 Genomes Project and GEUVADIS to identify potential causative variants that have impact on cardiovascular disorders and their risk factors
Next, our aim is to analyze the effects of the homozygous and compound heterozygous LoF variants on gene expression using RNA sequencing data from the +500 cohort and validate the positive signals in larger FINRISK cohorts (n~40 000).

PS18.41
Genetic polymorphism regulating the accumulation of soluble inflammatory mediators in platelet components (PCs)

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Introduction: Blood platelets destined for transfusion release panoply of inflammatory molecules during preparation and storage. The rationale of this study is to identify regulatory genetic polymorphisms that induce high levels of inflammatory molecules in PCs.
Methods: Supernatant from non-leukodepleted PCs was sampled from 150 PCs prepared for transfusion purpose. Luminex® technique was performed for measuring levels of sCD62P, RANTES, sCD40L, IL8, IL1β and TNFα (MILLIPLEX® MAP Kit Millipore). In parallel, Genomic DNA was extracted from every blood donor, after written consent, using FlexiGene® DNA kit (Qiagen, Paris, France).
Candidate polymorphisms were chosen from the HGMD® professional data base (http://www.hgmd.org/) and genotyped using Tetra-primer ARMS-PCR and RFLP-PCR. All of them were involved in transcription regulation.
Studied polymorphisms were as follow: rs6127 and rs6136 for SELP (CD62P gene); rs3092952 for CD40L; rs1126647 and rs4073 for IL-8; rs2280788 for RANTES; rs1799964 for TNFα and rs1143627 for IL-1β.
Results: We studied the evolution of the amount accumulated during the storage of each inflammatory molecule, depending on the genotype. Levels of sCD62P and IL-1β were genotype associated (p=0.04 and p=0.015, respectively). The G allele of rs6136 for SELP upregulated the concentration of sCD62P and the C allele of rs1143627 of IL-1β gene upregulated the concentration of IL-1β.
Conclusion: If confirmed in a larger study, we suggest using these polymorphisms to prioritize distribution of PCs in order to give products with lower concentration of inflammatory molecules. Exploring these polymorphisms could prevent adverse reactions for patients who previously developed transfusion inflammatory reactions.
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PM18.42
The influence of the genetic background on the causality of variants

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Next generation sequencing has revolutionized the identification of genetic variants. To be useful in a clinical setting, we need to distinguish causal from genuine variants. One can easily collect convincing but statistically poor evidence that supports the link between a variant and a disease, even if the variant has no true effect on the disease at all. The effect is even worse if a genetic diagnosis is independent of a current phenotype, as it is the case for the so-called incidental findings.
The genetic background has an influence on the pathogenicity of variants. Hence, we aim to assess the population specific patterns for disease-causing variants and the clinical relevance of variants that differ between populations.
We compared the distribution of reported disease-causing variants in the publicly available 1000 Genomes dataset. Principal component analysis on all variants listed in HGMD, ClinVar and based on the ACMG guidelines demonstrates strong population structure. We find variants that are causal across all populations; presumably only disease-causing in one population; and likely to be false positive.
Our work highlights several aspects. To assess the functional impact of a variant, we need to carefully match the ethnicity of the patient with the population in which the variant is reported. This is, however, difficult in admixed populations. We need to carefully evaluate each variant even if reported as causal. How reliable is the pathogenicity flag of a rare variant that is common in another population? How can we assess the causality if no functional data is available?

PS18.43
Regional variation in health-related traits in Scotland: genes or environment?

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Patterns of genetic variation provide valuable information for research on disease and epidemiological traits. Geography shapes genetic structure across European populations and geographic associations with health-related traits are known. Substantial disparities in health outcomes including lifespan exist within Scotland but evidence that genomic variation underpins these is limited. We analysed geographic stratification using Generation Scotland's Scottish Family Health Study (GS:SFHS), a cohort designed for study of complex traits and diseases. Principal component analysis of ~200K SNPs in approximate linkage equilibrium from a subset of 6909 unrelated individuals identified a significant correlation between genomic structure and the geographic origin of the individuals. Using information of the geographic origin of the four grandparents of each individual, we could predict more precisely the genomic origin of other individuals, providing an accurate map of Scotland. Traits including cholesterol and high-density lipoprotein had significant mean differences between regions in the GS:SFHS data. We utilised a linear mixed model including a genomic relationship matrix to separate the population's genomic structure from environmental effects of region of residence. The effects of region of residence on the traits remained

significant and largely unchanged by the inclusion of genetic relationships and genomic origin of individuals suggesting that the regional effects are of environmental, rather than genetic, origin. These results show the importance of including genetics in a linear mixed model context to disentangle the environmental effects, and point out the importance of the environment in health-related traits.

PM18.44

A genome-wide association meta-analysis of gastroenteritis in children

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Introduction: In Western countries the principal causes of childhood gastroenteritis are Rotavirus and Noravirus infections. Infection peak age is between 6 months and 2y. The heritability for gastroenteritis has been estimated in 54%. We performed a genome-wide association meta-analysis (GWAMA) to identify genetic variants for gastroenteritis susceptibility during first years of life.

Material and Methods: Six thousand children from 5 European cohorts from The EArly Genetics and Lifecourse Epidemiology (EAGLE) Consortium participated in the discovery phase. Gastroenteritis was defined based on questionnaire data or medical records at ages 1y and 2y. Gastroenteritis with or without clinical diagnosis were used as outcomes and followed in enrichment pathway analysis (MAGENTA-KEGG). Top signals from the discovery step will be replicated in additional 4,000 children from the consortium.

Results: Genetic variants near FUT2 gene were associated with gastroenteritis at age 1y (P<1.1E-09), yet some heterogeneity was observed among cohorts (P het= 0.03). FUT2 codes for a glycosyltransferase that participates in the histo-blood antigen production and is associated to infection susceptibility (including Rotavirus and Noravirus). Suggestive associated variants (P< 1E-05) mapped near A4GALT (histo-blood antigen), NPPA-NPPB (blood pressure), RYR2 genes (similarity to rotaviral VP6 protein), or close to GWAS signals for inflammatory bowel disease (PTER and IL17REL). Nominally associated pathways as Vibrio Cholerae infection, T cell receptor signaling, NOD like receptor signaling, glycosphingolipid biosynthesis and cell adhesion, among others, were identified.

Conclusions: Overall, sixty-four suggestive polymorphisms, including FUT2 locus, were identified in the discovery step and will be followed in additional samples.

PS18.45

Influence of genetic variants on heart failure occurrence and outcomes in an Italian population

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Introduction: Genetic factors can modify severity and progression of heart failure (HF). The CHARGE Consortium GWAS reported that the loci 15q22 and 3p22 are associated with occurrence and with risk of death in HF patients, respectively. Furthermore, a recent European GWAS tested the association of Dilated Cardiomyopathy (DCM) with 517,382 SNPs finding significant associations for rs2234962 and rs10927875. We focused on these conditions in order to replicate these results in an Italian population.

Materials and Methods: Our case-control study included 2535 HF patients from GISSI-HF study (728 have DCM phenotype) and 1196 healthy controls recruited among blood donors. All samples have been genotyped for rs12638540, rs10519210, rs2234962 and rs10927875 polymorphisms. Multivariable logistic regression analyses of the case-control were adjusted for age and sex whereas the association of the SNPs with study endpoints was adjusted for all the baseline variables associated with the outcome of interest.

Results: Association with a decreased risk of HF was obtained for rs10519210 G allele (p=0.03), rs10927875 T allele (p=0.002) and rs2234962 C allele (p=0.04). rs10927875 T and rs2234962 C alleles resulted associated with a decreased risk of DCM (p=0.006 and p=<0.0001, respectively). None of the SNPs provided association with ischemic aetiology of HF. As to the rs12638540 G allele, we confirmed its association with the combined endpoint of mortality and cardiovascular hospitalization (p=0.018), but not for all-cause mortality (p=0.42).

Conclusions: These data confirm that the genetic variants identified by the GWASs can affect the occurrence of HF as well as the outcome of HF patients

PM18.46

Genetic findings in babies whose meconium and colostrum samples containing heavy metals: an example from Kocaeli in Turkey

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Introduction: Exposure to air pollution and particulate matter with 10 micron diameters or less threat human lives significantly. Atmospheric deposits of heavy metals like Arsenic (As), Aluminum (Al), Cadmium (Cd), Copper (Cu), Mercury (Hg), Lead (Pb) and Zinc (Zn) could damage DNA and this damage results in chromosomal aberrations frequently. Heavy metals exposed mothers can transfer these metals to their babies through breastfeeding and circulating blood.

Materials and Methods: We investigated the presence of genetic alterations using array CGH analysis in 32 babies whose meconium and colostrum samples are containing heavy metals. Mothers of 24 babies were living in the industrial town, Dilovası and mothers of 8 babies were living in nonindustrial town, Kandıra.

Results: We found increased risks of genomic changes in heavy metal exposed mothers' babies compared to unexposed mothers' babies both in colostrum and in meconium. For the meconium samples, exposure to As and Zn increased the risk of aberrations (1.3-fold), and exposure to Al, Cd and Hg increased the presence of CNV (Al: 2.7-fold, Cd:1.4-fold, and Hg: 2.0-fold). In colostrum samples, exposure to Al, Cu, Cd and Pb increased the risk of the presence of aberrations (Al: 1.3-fold, Cd: 1.3-fold,Cu: 1.1-fold, and Pb: 3.6-fold), and the exposure to Cd and Zn increased the risk of the presence of CNV (Cd: 2.2-fold, Zn: 1.5-fold).

Conclusion: Our findings indicate that exposure to heavy metals investigated in the scope of this study can result in genomic variation either in the form of aberration or CNV.

PS18.47

Phenotypic expression of haemophilia B in patients with six novel F9 gene mutations

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Hemophilia B, X-linked recessive bleeding disorder, is caused by a wide range of mutations in the *F9* gene. It is characterized by deficiency in factor IX clotting activity. The disease is classified as severe, moderate or mild, and the phenotype severity is related to the type and position of the mutation in the *F9* gene.

During molecular characterization of hemophilia B in Macedonia six unique and unreported changes in the *F9* gene were revealed, confirming the high heterogeneity of molecular defects leading to disease. All mutations occurred in exons coding for the mature FIX domains. Three were missense mutations: c.536G>C,p.Gly179Ala; c.875A>C,p.Gln292Pro and c.1215T>G,p.Asp405Glu, while three were small deletions/insertions: c.230_231delTTinsA, p.Val77Asp*fs**27; delTA deletion affecting nucleotides c.849_850 leading to

Ile284*fs*, and c.1095delA or p.Ser365Ser*fs**3. Each novel missense mutation occurred at a highly conserved region. Prediction of the functional effects of the nucleotide changes was performed using in silico analysis.
All patients were severely affected with FIX<1%, and different episodes of bleeding into joints and muscles. Only the patient with c.875A>C,p.Gln292-Pro missense mutation had moderate form of disease (fIX 3%).
High titer of inhibitors, were registered only in the patient with deletion c.230_231delTTinsA. He was diagnosed at two years of age after thung injury, and since then more than 20 episodes of spontaneous bleeding, were registered. He developed high titer of inhibitors (20U), soon after starting prophylactic therapy.
In summary, these mutations may contribute for more precise identification of the structure-function relationship and understanding the nature of the factor IX molecule.

PM18.48
Heritability estimation from summary statistics using generalized estimating equations

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Introduction: In a recently published work, Bulik-Sullivan et al. (Nature Genetics, 2015) provide a methodological approach to differentiate between an inflation of test statistics in genome-wide association studies resulting from a polygenic architecture and from cryptic relatedness. This makes it also possible to estimate the heritability from summary statistics without requiring genotype data.
The approach of Bulik-Sullivan et al. estimates LD Scores from a reference panel and utilizes these quantities as covariates in a weighted linear regression of the squared test statistics. Since the test statistics are not independent of each other, a bootstrap method is applied to obtain robust standard errors.
Material and Methods: Building on the same mean model, our objective is to incorporate more useful extern information into the estimation in order to improve the efficiency of the estimation. In particular, we divide the genomic region into blocks of moderate size. For these blocks, the correlation structure between test statistics can be approximated by LD information from reference panels. Our estimation procedure is based on generalized estimating equations (GEE). We use the LD information to set up the working-correlation matrices for each block, whereas we do not require that nearby blocks are independent.
Results: We show that the GEE-related asymptotic results are still valid under reasonable assumptions. It is important to note that the working-correlation matrices are not required to be exactly the true correlation matrices in order to obtain consistent estimates and correct standard errors.
Conclusion: These results imply that our approach improves the heritability estimation framework.

PS18.49
The genetic ancestry of the Sherpa people

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Introduction: The Sherpa are an indigenous ethnic group of the Himalayas that display a remarkable ability to function at high altitude. Historical records suggest that the Sherpa migrated from Eastern Tibet to the Solu-Khumbu region of Nepal approximately 500 years ago. We set out to shed further light on the history of the Sherpa and other indigenous high altitude populations of the Himalayas through analysis of dense genomic data.
Methods: We had access to dense genotype data for 115 Sherpa from eight Sherpa-villages in the Khumbu region of Eastern Nepal. We also included 76 Nepalese individuals of seven different ethnicities and 91 individuals from two regions of Tibet; Lhasa and Yunnan. We complemented this dataset with 701 samples from neighbouring populations of the greater Himalayan region including individuals along the Silk Road; Kazakistan, Kirgykistan, Tajikistan and Uzbekistan, India, Pakistan and China. We conducted principal component, admixture and homozygosity analysis on this dataset.
Discussion and Conclusion: Our results suggest the Sherpa are a remarkably isolated, homogenous high altitude population. We confirmed the presence of an ancestral component that appears specific to high altitude populations of the Himalayas and is enriched in the Nepalese Sherpa, with particularly high proportions identified in individuals from a specific Sherpa village in the Khumbu valley of Nepal. Other indigenous populations of Nepal show varying degrees of admixture between southern (Indian), northern (Han) and Himalayan ancestry. Analysis of homozygosity in the Sherpa also indicate recent consanguinity within the population.
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PM18.50
GENETICS OF THE SPHINGOLIPID METABOLISM IN HYPERTENSION

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Essential hypertension refers to hypertension with no known cause accounting for 90-95% of all hypertensive subjects and is a major risk factor for stroke, heart disease, and end-stage renal disease. Hypertension is a polygenic disease and as such is supposed to harbour several genetic defects as the causative agents, but so far only a few potential genes have been identified. The major reasons for this modest progress are the unresolved physiologically heterogeneity of the disease and the prevailing monogenic approach to identify genes of importance. The physiologically heterogeneity was resolved by partition of the study population by combined latent class analysis and structural equation modelling into an ensemble of physiological more homogeneous subpopulations. Two-gene interactions were evaluated by variance decomposition and a new weighted mutual information score. The latter analytical approach considerably reduced the number of significant interactions detected by variance decomposition. It was established that the sphingolipid metabolic network is of significant importance in regulating the blood pressure.
In particular, acid ceramidase and sphingosine kinase 1 are the hubs collating the genetic information of blood pressure regulation.

PS18.51
Population Structure of the Island of Hvar - Maternal and Paternal Perspective

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Island of Hvar is situated in the central Eastern Adriatic, and its relatively small rural population has been reproductively isolated throughout history. Therefore, founder effects, genetic drift and inbreeding have had a significant role in the shaping of the current genetic diversity of Hvar Islanders. Island isolates are among the most suitable populations for theoretical analyses of microevolutionary processes and population differentiation. In this context, mitochondrial DNA (mtDNA) an Y chromosome studies are used to reveal demographic history, migrations, population origins and genetic structure of a population. We analyzed SNP markers of 161 mtDNAs and of 412 Y chromosomes from the island of Hvar, including STRs of 20 male islanders. Results showed a much higher number of mtDNA haplogroups (34) than Y chromosome ones (15). Haplotype diversity analysis showed low gene diversity among both mtDNAs and Y chromosomes on the island, while haplogroup distribution points to a significant population differentiation between the eastern and western part of the island. This substructure effect stems from different representation of subhaplogroups, as well as from differences between haplotypes within the same subhaplogroups and is based on specific demographic processes which occurred on Hvar during its history. When put in a wider context, haplogroups of both uniparental markers placed Hvar in the context of South-Eastern Europe, but the frequencies and the distribution of certain haplogroups significantly deviate from other SEE populations.

PM18.52

Insights into the genetic architecture of isolated populations through whole genome sequencing

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Isolated populations have population genetic characteristics that can boost power in genetic association studies of complex traits. Leveraging these advantageous characteristics requires an understanding of their population genetic parameters, such as effective population size, population split time and migration rate, which have shaped their sequence variation. Here, we perform a comprehensive investigation of these parameters using low-depth (4-8x) whole genome sequencing across multiple isolates: Orkney (n=399) from the UK, Friuli-Venezia Giulia (n=250) and Val Borbera (n=225) from Italy, Kuusamo (n=377) from Finland and HELIC-MANOLIS (n=249) from Greece. We compare these isolates to their nearest general populations; UK10K (n=3,781), TSI from the 1000 Genomes Project (n=108), SiSu cohorts (n=1,564, Finland) and TEENAGE (n=100, Greece). We find that all isolates are genetically close to their nearest general population, but have lower genetic diversity, longer LD blocks and a higher proportion of haplotype matching within individuals (runs of homozygosity), as expected because of genetic drift. We investigate the contribution of different population genetic parameters to these characteristics and construct an 'isolation index', which we show can also be calculated reliably from SNP genotype data. Populations with the highest isolation index scores have the greatest overall depletion of rare functional variants, but enrichment of relatively common ones; they also provide examples of positive selection with potential biological relevance. On this basis, candidate isolated populations can be evaluated for their informativeness in association studies.

PS18.53

The *KLOTHO* gene polymorphisms are associated with hypertension in the Chinese population of Matsu islands: a population-based follow-up study

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Introduction: Hypertension is a major risk factor for cardiovascular diseases, stroke, and end-stage renal disease. Hypertension has been ranked at the top ten leading causes of death in Matsu. The genetic contribution to blood pressure variation is estimated ranging from 30-50%. The *KLOTHO* gene was identified as an aging-suppressor gene and can regulate oxidative stress as well as protect vascular endothelial function, thus attenuate the progression of hypertension. To investigate the role of *KLOTHO* gene polymorphisms on hypertension, we conducted a community-based follow-up study in the Matsu islands of Taiwan.
Methods: From 2002 to 2009, 3707 subjects had participated in the Matsu Community-Based Integrated Health Screening project. We included 1955 Chinese subjects with DNA sample available and selected 6 SNPs on the *KLOTHO* gene (rs1207568, rs571118, rs577912, rs3752472, rs564481 and rs650439) for genotyping.
Results: We found the CT genotype carriers of rs564481 had a significantly higher risk of hypertension than CC carriers (OR = 1.40, 95% CI = 1.02-1.91) and the GGACTA carriers had a higher risk of hypertension than non-carriers (OR = 1.68, 95% CI = 1.16-2.43). We also found that the TT genotype carriers of rs564481 will significantly increase both SBP and DBP than the CC carriers over the study period. Besides, the SBP of the GGACTA haplotype carriers were significantly increased by 1.85 mmHg compared with their counterparts (p = 0.022) during the follow-up period.
Conclusion: Our findings provide evidence that the *KLOTHO* gene may play an important role on hypertension in Chinese population resident in Matsu.

PM18.54

Homozygous loss-of-function variants in European cosmopolitan and isolate populations

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Homozygous Loss of Function (HLOF) variants provide a valuable window on gene function in humans, as well as an inventory of the human genes that are not essential for survival and reproduction. All humans carry at least a few HLOF variants, but the exact number of inactivated genes that can be tolerated is currently unknown - as are the phenotypic effects of losing function for most human genes. Here, we make use of 1,432 whole exome sequences from five European populations to expand the catalogue of known human HLOF mutations; after stringent filtering of variants in our dataset, we identify a total of 173 HLOF mutations, 76 (44%) of which have not been observed previously. We find that population isolates are particularly well suited to surveys of novel HLOF genes because individuals in such populations carry extensive runs of homozygosity, which we show are enriched for novel, rare HLOF variants. Further, we make use of extensive phenotypic data to show that most HLOFs, ascertained in population-based samples, appear to have little detectable effect on the phenotype. On the contrary, we document several genes directly implicated in disease that seem to tolerate HLOF variants. Overall HLOF genes are enriched for olfactory receptor function and are expressed in testes more often than expected, consistent with reduced purifying selection and incipient pseudogenisation.

PS18.55

Molecular variation of three SNPs in LIN28B, related to developmental timing traits, in 24 Mediterranean populations

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The aim of this study is to screen, for the first time, the variation of the *LIN28B* gene in the whole Mediterranean region, through the analysis of the allele distribution of three single nucleotide polymorphisms (SNPs), namely rs7759938, rs314277 and rs221639, in 24 populations. These SNPs have been recently related to the age at menarche, pubertal height growth, peripubertal body mass index, levels of prenatal testosterone exposure and cancer survival. 1197 DNA samples were genotyped. The allele and haplotype frequencies were used to determine the relationships between populations, using also data from the 1000 Genomes Project for external comparisons. The genotype distributions and the population structure between populations and groups of populations were determined. The population results indicate a significant degree of variation (F_{ST}=0.043, p<0.0001). Allele and haplotype frequencies show significant differences among populations. A hierarchical variance analysis is consistent with a main differentiation between the populations on the two North and South coasts of the Mediterranean. This difference is especially evident in the unexpected distribution of the SNP rs221639, which shows one of the highest F_{ST} (11.5%, p<0.0001) values described so far in the Mediterranean region. The population differentiation and the structuring of the genetic variance, in agreement with other previous studies, indicate that the SNPs studied are good tools for the study of human populations, even at a microgeographic level.
This study was supported by the research project CGL2011-27866 of the Ministry of Science and Innovation of Spain.

PM18.56

Whole genome sequences are required to fully resolve the linkage disequilibrium structure of human populations

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An understanding of linkage disequilibrium (LD) structures in the human genome underpins much of medical genetics, and provides a basis for disease gene mapping investigating biological mechanisms such as recombination and selection. Whole genome sequencing (WGS) provides the opportunity to determine LD structures at maximal resolution. We compare LD maps produced utilising WGS data with LD maps produced utilising the HapMap dataset, for representative European and African populations. Maps derived from the WGS data achieve much greater resolution of LD structure (~ 5 fold), which translates to improved utility. The absence of ascertainment bias in variant genotyping improves the population representativeness of

the maps, and highlights the extent of uncaptured variation present in African populations using array genotyping methodologies. Given the extended time to an effective population bottleneck for African populations, larger sample sizes and higher marker densities are required to fully resolve the LD structure. Whole genome LD maps will provide a rich resource for medical genetics, as well as for recombination biology.
This work is funded under the UK Biotechnology and Biological Sciences Research Council (BBSRC) 'Excellence with Impact' scheme.

PS18.57
The influence of lipoprotein lipase gene polymorphisms on postprandial lipoprotein metabolism

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Lipoprotein lipase (LPL) is a key rate-limiting enzyme for the hydrolysis of triacylglycerol (TAG) in chylomicrons and VLDL. Polymorphisms in the LPL gene have been previously demonstrated in association with several lipid phenotypes; however, the findings have been inconsistent. Given that postprandial assessment of lipoprotein metabolism may provide a more physiological perspective of disturbances in lipoprotein homeostasis compared to assessment in the fasting state, we have investigated the influence of three LPL polymorphisms (rs268, 291S; rs320, HindIII; rs328, S447) on postprandial TAG, non-esterified fatty acid (NEFA), glucose and insulin in 261 participants using a sequential meal challenge consisting of a mixed breakfast (0 min; 49g fat) and lunch (330 min; 29g fat). Blood samples were taken at 60 min intervals until 480 min after the test breakfast. At baseline, LPL S477 allele carriers had higher TAG (P=0.05) and lower HDL-C (P=0.02). Following the sequential meal challenge, there was an association of LPL S447 polymorphism with postprandial TAG and glucose, where S477 allele carriers had 12% and 8.4% higher TAG area under the curve (AUC) (P=0.04) and glucose AUC (P=0.006), respectively. In addition, a borderline association was observed between S477 and postprandial NEFA levels (P=0.05). With LPL 291N polymorphism, those who were homozygous for N allele had higher fasting NEFA (P=0.038) and postprandial NEFA response (P=0.003). In conclusion, our data suggest that carriers of 291S and S447 polymorphisms have an abnormal postprandial response to sequential meal challenge and provide further in vivo evidence as to the functional nature of LPL polymorphisms.

PM18.58
Large scale genotyping key to hidden genetic diversity of the population of Lithuania

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Previous studies showed gradient of genetic diversity of mtDNA and Y chromosome genetic markers within the population of Lithuania. Further, genetic diversity of autosomal genetic markers has been analyzed.
Venous blood samples and written consent forms were collected across six ethno-linguistic groups of Lithuania (North, South, West Žemaitija and East, South, West Aukštaitija). 253 samples were genotyped using the HumanOmniExpress-12v1.1 arrays (719,666 SNPs). After a systematic primary and secondary QC of the generated data, 590,665 autosomal SNPs and 188 samples remained for the subsequent analyses.
An MDS analysis and PCA based on 105,387 autosomal SNPs (pairwise LD r2<0.2) were performed. No obvious sets of clusters were seen in the scatter plot of MDS of all six ethno-linguistic groups of Lithuania. Though two clearly apparent clusters were detected in the scatter plot of MDS analysis when only samples from North Žemaitija and South Aukštaitija were included. No apparent clusters were seen in the scatter plot of MDS analysis including samples from Žemaitija only. Samples from South Aukštaitija, East or West Aukštaitija and North Žemaitija in the scatter plot of MDS analysis were positioned according to their North-South locations in the territory of Lithuania.
PC1 explained 0.61% (Tracy-Widom test, P<0.05) and PC2 - 0.59% of genetic variation among 188 samples of the Lithuanian population. According to the scatter plot of PCA, the greatest differentiation was detected among samples from the northwest and southeast of the population of Lithuania.
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PS18.59
A clinicopathological and genetic study of lynch syndrome in Morocco

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Introduction: Hereditary non polyposis colorectal cancer is an inherited disease with deleterious germline mutations in the DNA mismatch genes causing the development of colon cancer and other malignancies.
Purpose: This study aimed to characterise the mutation profile of MLH1 and MSH2 in Moroccan colorectal cancer cohort. This is the first study in Morocco screening the population of our colorectal cancer patients in order to identify the prevalence of the disease.
Methods: From January 2010, 214 newly diagnosed colorectal cancer tumors received immunohistochemical staining for MLH1 and MSH2. If any stain was absent, the medical genetics service were alerted for a genetic consultation, and a Sequencing of corresponding MMR genes (mlh1 and msh2) was performed using the sanger method.
Results: There were 21 (9.8%) cases with abnormal immunohistochemical results. Genetics was able to contact 19 (90.4%) of these patients, only 16 (76.1%) of them made an appointment. All the cases underwent the genetic testing of mlh1 and msh2 genes, wich was informative in four (25%) of the patients. The diagnosis of lynch syndrome were confirmed among two (0.9%) patients harbouring deleterious mutations, however, the other two cases were found to have mutations with uncertain significance.
Conclusion: This study attempted to define the frequency of lynch syndrome among Moroccan population. The preliminary results of the study showed that 0.9% were found to carry germline mutations of the mlh1 msh2 genes.
References: Lamberti C, Mangold E, Pagenstecher C et al (2006) Frequency of hereditary non-polyposis colorectal cancer among unselected patients with colorectal cancer in Germany. Digestion 74:58-67

PM18.60
Human coding variants in 2,628 Dutch individuals; the Rotterdam Study

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We performed whole-exome sequencing (Nimblegen v2, Illumina sequencing to mean depth of 55x) of a random sample of 3,000 Dutch Caucasian elderly men and women. Single nucleotide variants (SNVs) and small insertions and deletions (Indels) were called using the Genome Analysis Toolkit's (GATK) HaplotypeCaller and after QC and filtering a final dataset of 2,628 individuals was created. We detected 258,702 SNVs and 15,230 Indels with >2 alleles. Approximately 70% of SNVs and 50% of Indels were found reported in the ESP6500 database, with high concordance in minor allele frequency (MAF) (correlation of 0.97 and 0.91, respectively). 475 novel SNVs with MAF>20% were identified, which have not been described in any public database (1000G, ESP, GoNL & UK10K). Pathway analysis of these novel common SNVs showed significant enrichment for olfactory (p=1.4E-05) and taste transduction pathways (p=1.6E-03). As compared to the mean variant density of the genome (1 SNV in 448 nucleotides of coding sequence), we observed 18 enriched pathways among the 500 genes with the lowest SNV density, most of which were signaling or cancer pathways. Noticeably, MAPK1 (1 SNV in 1,400), PIK3CB (1 SNV in 1,305) and PIK3R1 (1 in 1,761) were involved in many pathways, suggesting key regulatory functions for these genes. Because of broad and deep longitudinal phenotyping of the Rotterdam Study cohort, this dataset is currently explored for rare genetic variants in relation to complex traits and diseases as well as known variants in typical Mendelian diseases, and is accessible as a control dataset.

PS18.61
Identification and functional study of a heterozygous missense mutation in UMOD gene in an Italian family affected by medullary cystic kidney disease type II.

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INTRODUCTION

The medullary cystic kidney disease type II (MCKD2) is an autosomal dominant disorder characterized by early onset of hyperuricemia, decreased fractional renal urate excretion and progressive interstitial nephropathy leading to end-stage renal disease. MCKD2 is caused by mutations in the UMOD gene, which encodes Uromodulin, a glycosylphosphatidylinositol-anchored protein that is expressed in the thick ascending limb of the loop of Henle and excreted in the urine. Over 90% of UMOD mutations are missense, and 62% alter a cysteine residue, implicating a role for protein misfolding in the disease. In this study we investigated the presence of mutations in UMOD gene in four members of Italian family showing the phenotype of MCKD2.

METHODS: Genomic DNA was extracted from peripheral blood leukocytes by using the Wizard Genomic DNA Purification kit following the manufacturer's instructions. Mutation analysis of the UMOD gene was performed by polymerase chain reaction and direct sequencing. A mutant UMOD construct, containing the identify mutation was created by in vitro mutagenesis. Transient transfection studies were performed in human embryonic kidney cells. Expression was evaluated by reverse transcription polymerase chain reaction (RT-PCR), western blot and immunofluorescence.

RESULTS: Sequence analysis revealed a heterozygous missense variation (c.187T>C; p.Cys63Arg) in exon 3 that altered an evolutionary conserved residue in the UMOD. Functional studies showed that the mutant protein was retained in the endoplasmic reticulum and was not excreted to the cell medium, as opposed to the wild-type protein.

CONCLUSIONS: Collectively, our results suggested that the variant may be the causative mutation in this family.

PM18.62

Effect of ACE I/D polymorphism on childhood obesity

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Obesity is a multifactorial disease influenced by genetic and environmental factors. The common insertion/deletion polymorphism (I/D) in the gene for angiotensin-converting enzyme (ACE) was previously associated with obesity, both in preschoolers, adolescents and adults. ACE I/D polymorphism might play a role in the development of the obesity and the hypertension, which are closely linked to cardiovascular risk factors

The aim of this study was to investigate the associations of obesity and ACE polymorphisms in patients with overweight and obesity. We enrolled 80 consecutive patients and 100 healthy subjects in the study. Both the obese cases and the normal-weight controls underwent the identical subset of standardized examinations. ACE insertion (I)/deletion (D) genotype was determined by PCR. The most common genotype in the obesity group was ID, with a lower percentage of DD and II (46%, 38.9% and 15.10% respectively). We did not find any difference between gender and genotypes in obese and control group. We found that 40% of children with moderate obesity have ID genotypes, while II genotype was most frequent (34%) in the severe obese subgroup. Children carrying I allele had a higher risk of obesity than those with allele D compared with the control group ($p<0.05$). These data suggest that ACE gene polymorphisms may influence the development of weight gain.

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PS18.63

Suggestive linkage to chromosomal regions 13q13.3 and 21q22.2 in families with Multiple Sclerosis

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Introduction: Multiple Sclerosis (MS) is an immune-mediated, inflammatory, demyelinating and neurodegenerative disease of the central nervous system with a proven, yet not fully established heritability. The objective of this study is to investigate the genetic basis of familial MS in Turkish population using a genome-wide linkage analysis in 10 multiply affected MS families from Turkey.

Materials and methods: Here we investigated linkage with MS across the genome using single nucleotide polymorphism (SNP) genotyping on the Illumina CytoSNP 300K array in 35 individuals from 10 MS families. Non-parametric linkage (NPL) scores were calculated for each of 3118 SNP markers spaced at an average of 1 cM intervals using SimWalk multipoint NPL analysis. Subsequent fine mapping of regions showing higher NPL scores was performed.

Results: The 10 families in which one or more individuals had MS showed suggestive evidence of linkage (NPL scores above 1.7) to chromosomal regions of 13q12.2-14.11 and 21q22.12-22.3. Fine mapping of these regions revealed that the most promising loci for linkage were mapped to 13q13.3 and 21q22.2, with NPL scores of 1.82 and 1.85, respectively. Inflammation or neurodegeneration associated genes some of which previously implicated in MS including HMGB1, POSTN, OLIG1, IFNAR1, IFNAR2 genes and also promising novel genes yet to be identified like N4BP2L2 and TRPC4 were found within the suggestive regions.

Conclusions: Although there was no significant evidence for linkage, our suggestive linkage results provide a framework for deep sequencing to identify new susceptibility genes and novel variants associated with risk of MS.

PS18.65

Genetic evidence for an origin of the Armenians from Bronze Age mixing of multiple populations

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The Armenians are a culturally isolated population who historically inhabited a region in the Near East bounded by the Mediterranean and Black seas and the Caucasus, but remain underrepresented in genetic studies and have a complex history including a major geographic displacement during World War One. Here, we analyse genome-wide variation in 173 Armenians and compare them to 78 other worldwide populations. We find that Armenians form a distinctive cluster linking the Near East, Europe, and the Caucasus. We show that Armenian diversity can be explained by several mixtures of Eurasian populations that occurred between ~3,000 and ~2,000 BCE, a period characterized by major population migrations after the domestication of the horse, appearance of chariots, and the rise of advanced civilizations in the Near East. However, genetic signals of population mixture cease after ~1,200 BCE when Bronze Age civilizations in the Eastern Mediterranean world suddenly and violently collapsed. Armenians have since remained isolated and genetic structure within the population developed ~500 years ago when Armenia was divided between the Ottomans and the Safavid Empire in Iran. Finally, we show that Armenians have higher genetic affinity to Neolithic Europeans than other present-day Near Easterners, and that 29% of the Armenian ancestry may originate from an ancestral population best represented by Neolithic Europeans. These results illustrate the benefits of combining genetic and archaeological information for understanding present-day populations.

PM18.66

Nitric oxide synthase genes and cardiovascular risk in Spain

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This is a case-control study of genetic association between the molecular variation in the nitric oxide synthase (NOS) genes and susceptibility to cardiovascular disease in Spanish population. Nitric oxide (NO) is an important physiological messenger synthesized by the three NOS isozymes, the neuronal NOS (NOS1), the inducible NOS (NOS2) and the endothelial NOS (NOS3). Potential genetic associations were assessed for different polymorphisms: 67 single nucleotide polymorphisms (SNP), 10 short tandem repeat polymorphisms (STRP) and 6 haplotypes on areas that showed high linkage disequilibrium, on all three NOS genes. These analyses were performed in a total of 903 samples: 408 heart disease patients and 495 non-affected, coming from two different sample designs, nuclear families (trios) and case-control. The association analysis was implemented by using the LAMP software which carries out the analysis combining case-control and trio familiy samples.

No haplotype significant association was found. The SNP results showed some significant associations, however when multiple testing correction (Bonferroni) was applied, all these associations became non-significant. The STRP results showed 7 out the 10 polymorphisms analysed significantly associated to heart disease. After multiple testing corrections 5 of them still showed significant associations. Most significantly associated polymorphisms were located on regulatory regions on the NOS1 and NOS2.

In conclusion only the variability in NOS1 and NOS2 genes appears to be significantly associated to heart disease in the Spanish population.

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PS18.67

Population variation of NOS genomic regions and coronary incidence differences in Europe

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Nitric Oxide Synthases (NOS) genes encode for key enzymes on nitric oxide (NO) availability, a substance involved in several cardiovascular processes. Although the variation in the NOS genes has been repeatedly tested in epidemiological studies, it has not systematically analyzed at population level. Population distribution of NOS markers and its relationship with population coronary incidence can shed light on the demographic processes that shape both the population distribution of cardiovascular-related genetic regions and coronary incidence variation among populations. In this study, 114 single nucleotide polymorphisms (SNP) and 17 tandem repeat polymorphisms (TRP) from NOS regions were determined in 1321 individuals from 31 populations from Europe, North Africa and the Middle East. The found variation was tested for selection, used to obtain inferences of population structure, and correlated with coronary mortality in 11 of these European populations. Our results on NOS variation support i) the absence of clear signs of selection for genetic variants elsewhere associated with cardiovascular diseases, and ii) the presence of a continuous genetic pattern among European and North African populations without a Mediterranean barrier for gene flow. The population structure NOS correlated with CAD mortality, explaining a remarkable proportion of coronary event rates (39-98%) among European populations. These results reinforce the hypothesis that genetic bases of cardiovascular diseases shows a geographical distribution mainly associated with random and/or demographic processes.

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PM18.68

Evolution of nuclear mitochondrial insertions in the genomes of primates

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The transfer of mitochondrial genetic material into the nuclear genomes of eukaryotes is a well-established, ongoing phenomenon. The recent advancement of high throughput sequencing technologies has enabled the interrogation of genomic variation from whole genome sequence data at a fine scale and allowed for the identification of other classes of structural variation that had been previously difficult to ascertain, including polymorphic nuclear mitochondrial insertions (numts). We have developed an approach to discover and genotype these numts using paired-end, next generation sequencing data and have previously described its application to human populations. Here, we extend our analysis to the whole genomes of 77 non-human primates consisting of chimpanzees, bonobos, gorillas and orang-utans. We report the identification of 476 novel numt insertions that segregate within each species, the majority of which were discovered in chimpanzees. We characterized and where possible have assembled their underlying sequences, and further examined these variants for biases in sequence context at insertion sites and their potential for functional effects. In addition, we also examined the patterns and origin of these insertions and have estimated their age. We investigated whether there are species-specific preferences for both polymorphic and fixed numt insertions and whether these are associated with the difference in the mutational load of numts observed between chimpanzees and the other species, including humans. To our knowledge, this is the first study to look at polymorphic numt insertions in non-human primates and we believe this research will help clarify to what extent that numts play a role in hominoid diversification and phenotypic variability.

PS18.69

The study of the FTO rs9939609 and ADRB3 rs4994 gene polymorphisms in association with obesity in a Romanian cohort of obese subjects

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Introduction: The human population worldwide faces an alarming increase in the incidence of obesity in all races, sexes, social, cultural, religious or geographic environments.Purpose: Verifying the FTO rs9939609 and ADRB3 rs4994 genetic variants frequencies in our cohort and their association with obesity. Setting-up an early detection test for the predisposition for this disorder in our country would be an important step in stopping its development. Material. Methods: The selected cohort for genotyping consisted of 64 subjects, 44 obese and 20 controls (33 adults, 23 children, 8 Prader-Willi-syndrome patients*).* The results were tested in correlation with obesity parameters and associated disorders. Results: This is a first approach of genotyping in a Romanian cohort comprising adults and children for two genes related both to energy accumulation and expenditure. The FTO allele A was more associated with obesity in adults than in children (statistical for both). The FTO rs9939609 polymorphism is a common SNP in our study group, its mutant A allele showing higher frequencies than worldwide. The ADRB3 polymorphism is a rare variant: its C allele proved correlations, some statistical, with obesity susceptibility, although no homozygous mutant subjects were reported. The rs4994 frequency was higher in our groups than worldwide. ADRB3 indicated higher associations with obesity predisposition in children. Prader-Willi subjects showed high frequencies of both polymorphisms. Conclusions: The study revealed to be highly populational and not completely in concordence with reported literature. A greater number of subjects and more obesity associated polymorphisms are needed for determining a Romanian obesity gene map and genetic susceptibility test.

PS18.71

New methods for family based studies identify genetic regions underlying oral cleft risk

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Oral clefts represent more than half of all craniofacial malformations, with an overall prevalence of about 1 per 600-700 live births worldwide. While there is substantial variation in birth prevalence across populations, oral clefts demonstrate strong familial aggregation and relatives of affected individuals are at significantly increased risk compared to the general population. Genome-wide association studies have shown numerous genes (including IRF6, ABCA4 and MAF) and even regions with few or no known genes (such as 8q24) play an important role in the etiology of oral clefts. However, much of the observed familial aggregation and heritability of oral clefts remains unexplained.

Large-scale sequencing in extended multiplex families can help to define the relationship between disease and genetic variants too rare in the population to be detected through tests of association in conventional study designs. Case-parent trio designs guard against confounding due to population stratification in tests for linkage and association with common markers, and also allow for the assessment of de-novo events and parent-of-origin effects. Both approaches can be readily adapted to test if structural variants also control risk to oral clefts.

We present the results from applying newly developed statistical methods and software for nucleotide and DNA copy number variants to data from family-based studies of oral clefts in subjects of Asian and European descent, identifying several novel genes and regions (including ADAMTS9, CDH1, and 7p14.1) as potential candidates underlying the etiology of oral clefts.

PM18.72

Association of the MMPs genes single nucleotide polymorphisms with gastric and duodenal ulcer in Volga-Ural region of Russia

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Peptic ulcer disease (PUD) refers to painful sores or ulcers in the lining of the stomach (GU) or duodenum (DU). Recent studies have indicated that gastric ulceration is associated with cleaving and remodeling of the extracellular matrix (ECM) by matrix metalloproteinases (MMP). The aim of this study was to investigate the allele and genotype distribution of SNPs in the MMP1 (rs2276109, rs4994379), MMP2 (rs2285053), MMP3 (rs3025053), MMP9 (rs3918242, rs17576), MMP12 (rs2276109) and metalloproteinase

tissue inhibitors TIMP2 (rs8179090), TIMP3 (rs9619311) genes in patients with PUD and healthy donors from Volga-Ural region of Russia.
The patient group consisted of 353 individuals with PUD, the control group included 285 unrelated non-ulcer individuals with different ethnic origins (Russians, Tatars, Bashkirs). Genomic DNA was extracted from peripheral blood leucocytes by standard phenol/chloroform method. Genotyping was performed by PCR-RFLP analysis.
The analysis shows that genotypes rs494379*A/G, rs17576*A/G are associated with the risk of PUD in Tatars (P=0.001;OR=2.37 and P=0.003;OR=2.19, respectively). Genotypes rs494379*A/G, rs17576*A/G are also markers of the increased risk of PUD development in H.pylori-positive individuals (P=0.007;OR=2.29 and P=0.009;OR=2.23, respectively), whereas rs3025053*5A/6A was protective (P=0.03;OR=0.43).
Thus, we have determined statistically significant association between MMPs genes polymorphisms and peptic ulcer in Volga-Ural region of Russia.

PS18.73
Utilizing the Jaccard index to reveal population stratification in sequencing data: A simulation study and an application to the 1000 Genomes Project

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Population stratification is one of the major sources of confounding in genetic association studies, potentially causing false-positive and false-negative results. Here, we present a novel approach for the identification of population substructure in high density genotyping data/next generation sequencing data. The approach exploits the co-appearances of rare genetic variants in individuals. The method can be applied to all available genetic loci, does not require linkage disequilibrium (LD) pruning, and is computationally fast. Using sequencing data from the 1000 Genomes Project (phase 3), the features of the approach are illustrated and compared to existing methodology (i.e. Eigenstrat). We find that our approach works particularly well for genetic loci with very small minor allele frequencies. The results suggest that the inclusion of rare-variant data/ sequencing data in our approach provides a much higher resolution picture of population substructure than it can be obtained with existing methodology. Furthermore, in simulation studies, the proposed methodology maintains the type 1 error accurately and shows higher power in some scenarios.

PM18.74
New putative regions of positive selection based on a sample of Mexican indigenous population

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The simultaneous genotyping of hundreds of thousands of SNPs in previously uncharacterized populations is of great importance given its potential to detect signals of recent positive selection as well as to depict insights of their genetic structure and their relationship to other human populations. To this end, we analyzed 442,450 SNPs in 469 individuals from four indigenous populations throughout Mexico (167 Nahuas, 103 Mayas, 98 Totonacs and 101 Zapotec). We calculated Fst, IHS an XP-EHH statistics to detect possible footprints of positive selection using two sets of data representing Asian and European population as outgroups, focusing on signals that relate to metabolic traits. Based on genetic differentiation, when Indigenous and European populations are considered, preliminary results clearly identified EDAR and SLC45A2 genes. In addition to this a 60 kb region was identified in chromosome 15 involving SLC28A2 gene. When comparing Indigenous and Asian population's three strong signals in chromosomes 14, 20 and 22 were found. Of particular interest is the result obtained in chromosome 14 where a 2 Mb region is detected including two long intergenic non-protein coding RNA and MDGA2, a gene previously associated to metabolic traits in GWAS. Further studies are being design to confirm these results to better understand their biological meaning.

PS18.75
Increased expression of proinflammatory signal transducer TLR4 receptor is associated with preterm birth

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Introduction- Toll-like receptor-4 (TLR4) is the major endotoxin-signaling receptor and is crucial for the initiation of immune response. Inflammation within the uterus has been implicated in the pathogenesis of preterm birth. In the present study, we reveal the possible implication of TLR4 expression and function for immunologic immaturity and vulnerability of premature and even mature newborns. However, little is known about their expression and regulation in inflammatory pathway. Therefore, we aimed to assess the association of expression of TLR-4 in mothers with preterm birth.
Material and methods- A total of 135 mothers each who delivered preterm and term births were included. Total RNA was isolated from blood of all subjects and the quantification of TLR-4 expression was measured by real time PCR. The18SrRNA gene was used as an endogenous control (housekeeping gene).Results were evaluated using the delta-delta Ct method, where delta Ct was calculated as (TLR4 Ct) (18SrRNACt), and the relative quantity of TLR4 mRNA expression was calculated by the delta-delta Ct as 2-[case sample delta Ct)− (control sample delta Ct)].
Results-TLR4 mRNA expression was found to be 2.5 ±0.08 fold higher in cases (TLR4 mRNA levels of 0.72 ± 0.037) than in controls (TLR4 mRNA levels 0.498 ± 0.038, p=0.0002).
Conclusion-We concluded that the expression of TLR4 mRNA was elevated in mothers with preterm birth. It may be used as a biomarker associated with preterm labor.
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PM18.76
Identification of rare disease codes in ICD-9-CM and exploration of Minimal Hospital Data

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For financial reimbursement, Belgian hospitals need to comply with compulsory registration of the Minimal Hospital Data (MHD). These comprise various data on all hospital admissions at patient level. Our ultimate goal is to query this comprehensive repository to answer various health care related questions in the area of rare diseases (80% of which are genetic diseases).
Unfortunately, such analyses are hampered by lack of a suitable codification/classification system in the hospital information systems. Although efforts are being made to introduce the ORPHA rare disease codes, diagnostic information in the MHD was coded with ICD-9-CM. Since January 2015, ICD-10-CM is in use .
In order to investigate the problem of rare disease coding more thoroughly and to enable the exploitation of historical data, we aimed to identify all codes of interest in the ICD-9-CM and ICD-10-CM. Mapping and linearization of the ORPHA codes to ICD-10-CM codes is done by Orphanet (INSERM) and available at orphadata.org. ICD-9-CM/ ICD-10-CM conversion tables were created by the FPS. By standardizing and combining these lists we distilled a final set of ICD9-CM codes for rare diseases of equivalent, broader as well as narrower terms.
We conclude that for rare diseases, the transition to ICD-10-CM was indispensable due to a higher degree of granularity. We will discuss the mapping methodology, its accuracy and results. In addition, we will illustrate the value of MHD analyses for identification of rare diseases by presenting information derived from MHD for a representative rare disease.

PS18.77
Rare variant association tests for longitudinal family studies

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Identification of rare variants has been shown to be critical in dissecting the disease etiology for they are often functional relevant. Family studies enriched with affected members are likely to aggregate functional variants, thus, increase statistical power for rare variant detection. Longitudinal family studies further provide additional information for identifying genetic and environmental factors associated with disease over time. However, rare variant analysis methods of longitudinal family data remain fairly li-

mited, which need to account for different sources of correlations as well as to handle large sequencing data efficiently. To identify rare variants in longitudinal family studies, we extend the powerful pedigree-based burden and kernel association tests for genetic longitudinal studies. The proposed methods model phenotypes following a distribution in the exponential family. Confounding factors as fixed effects can be adjusted for. These tests account for the complex correlations between repeated measures from the same phenotype (the serial correlation) and between individuals within the same family (the familial correlation). We use generalized estimating equation (GEE) approaches to generalize the pedigree-based burden and kernel tests to multiple correlated phenotypes under generalized linear model framework. Comprehensive simulation studies were conducted to compare the proposed tests with marginal models using GEE and mixed effects models under a variety of configurations. The proposed tests are applied to the Diabetes Heart Study (DHS). Significant exome variants of POMGNT1 and JAK1 genes associated with type 2 diabetes are identified.

PM18.78

Strategies to improve the performance of rare variant rare disease association studies by optimizing the selection of controls

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When analyzing a case groups of patients with ultra-rare disorders the ethnicities are often diverse and the data quality might vary. The population substructure in the case group as well as the heterogeneous data quality can cause substantial inflation of test statistics and result in spurious associations in case-control studies if not properly adjusted for. Existing techniques to correct for confounding effects were especially developed for common variants and are not applicable to rare variants. We therefore analyzed different strategies to select suitable controls for cases that originate from different populations and differ in data quality. We developed an approach to build up a control group that is most similar to the individuals in the case group with respect to ethnicity and data quality by means of a metric that puts more weight on rare variants. We simulated different disease entities on real exome data and show that a similarity-based selection schemes can help to reduce false-positive associations and to optimize the performance of the statistical tests. We reanalyzed collections of unrelated patients with Kabuki make-up syndrome, Hyperphosphatasia with Mental Retardation syndrome and Catel-Manzke syndrome for which the disease genes were recently described. We show that rare variant association tests are more sensitive and specific in identifying the disease gene than intersection filters and should thus be considered as an favorable approach in analyzing even small patient cohorts.

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PS18.79

Genetic variations in Interleukin genes and recurrent spontaneous abortions

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Indroduction: Inflammatory cytokine cascades have been implicated in the pathogenesis of pregnancy loss. Polymorphisms in cytokine genes may affect the risk of recurrent spontaneous abortions (RSA) but genetic association studies have often contradictory results among different populations. The purpose of this study was to investigate whether polymorphisms in the interleukin genes IL-6 and IL-1 are implicated in pregnancy outcome.
Methods: A total of 220 Greek women were examined for the IL-6 -174 T/C and IL-1 Ra polymorphisms. The patient group consisted of 120 women with at least three unexplained recurrent spontaneous abortions before 20 weeks of gestation while 100 women with at least two live births and without history of abortions served as controls. All individuals were genotyped by the PCR-RFLP method, for the first polymorphism, and by PCR method for the second polymorphism.
Results: The data between the two groups were analyzed by chi-square test or Fisher's exact test. Our results indicated that for both studied polymorphisms there are no significant differences in genotype or in allele frequencies between the patient and the control group.
Conclusion: Conversely to some other populations, in our Caucasian population the polymorphisms IL-6 -174 T/C and IL-1 Ra are not associated with the risk for RSA.

PM18.80

BMD loci underlie developmental determination of ethnic differences in skeletal fragility across populations due to selection pressures

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Bone mineral density (BMD) is a highly heritable trait used both for the diagnosis of osteoporosis in adults and to assess bone health in children. Ethnic differences in BMD have been documented, with markedly higher levels in individuals of African descent, which partially explain disparity in osteoporosis risk across populations. To date, 63 independent genetic variants have been associated with BMD in adults of Northern-European ancestry. Here, we demonstrate that 61 of these variants are predictive of BMD early in life by studying their compound effect within two multiethnic pediatric cohorts. Furthermore, we show that within these cohorts and across populations worldwide the frequency of those alleles associated with increased BMD is systematically elevated in individuals of Sub-Saharan African ancestry. The amount of differentiation in the BMD genetic scores among Sub-Saharan and non-Sub-Saharan populations and together with neutrality tests, suggest that these allelic differences are compatible with the hypothesis of selective pressures acting on the genetic determinants of BMD, providing a new example of polygenic adaptation in a human trait.

PS18.81

The regional analysis of Slovenian mitochondrial gene pool reveals sharp gradient of J1c haplogroup

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Introduction: The Slovenian territory, which is geographically positioned in central Europe between the Alps, the Adriatic Sea, Pannonian basin and Dinaric Mountains, has experienced turbulent historic events, which are reflected in different archaeological cultures found in the area. Turbulent historic events and diverse geography of the region have produced a diverse contemporary population whose genetic analysis could provide insight into past demographic events.
Materials and Methods: In order to characterise the Slovenian mitochondrial gene pool at the micro-geographic level and to compare it with surrounding populations we analyzed a total of 402 individuals from five Slovenian regions, by typing HVR I, HVR II and coding region polymorphisms of mtDNA.
Results: Analysis revealed 47 haplogroups and subhaplogroups, the most common of which were H*, H1, J1c, T2 and U5a. The intra-population comparison revealed a sharp gradient of J1c haplogroup between different Slovenian populations, with the peak frequency of 24.5 % in the population of Littoral Region. The high frequency of J1c haplogroup among the population of Littoral Region could represent the genetic trace of the early Neolithic expansion along the East Adriatic coastal region, which is in line with the archaeological horizon known as an Impressed Ware culture, whose findings are characteristic for Slovenian Littoral Region.
Conclusion: These results outline not only the impact of early Neolithic genetic input on a modern Slovenian genetic structure, but also an importance of regional sampling strategy for the understanding of historical demographic events which define the history of East Adriatic and Central European populations.

PM18.82

Folic acid supplementation influences the distribution of neural tube defect subtypes: a registry-based study

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Objective: To study the effect of folic acid supplementation on the distribution of neural tube defect (NTD) subtypes.
Design: Registry-based, case-only study.
Setting: Northern Netherlands, 1997-2012.

Population: Live births, stillbirths, miscarriages and terminated pregnancies due to fetal anomaly with an NTD registered by EUROCAT Northern Netherlands with birth years 1997-2012. After excluding cases with unspecified NTD location, unknown maternal folic acid supplementation status, or a probable syndromal or chromosomal cause for the NTD, we had 173 cases in our study population.
Methods: Chi-square testing was used to assess differences in NTD subtype distribution between two groups: correct folic acid supplementation and incorrect or no folic acid supplementation. We then assessed whether exposure to the main risk factors for NTD influenced the NTD subtype distribution.
Main Outcome Measures: The proportion of anencephaly, cervical/thoracic spina bifida, lumbar/sacral spina bifida, and encephalocele per folic acid group.
Results: Proportionally fewer cervical/thoracic cases and more lumbar/sacral spina bifida cases were present in the correct folic acid supplementation group than in the group with incorrect or no folic acid supplementation, and this was irrespective of the presence of the main NTD risk factors. The effect on NTD subtype distribution was only seen when folic acid supplementation was started before conception.
Conclusions: Folic acid not only prevents the occurrence of a significant proportion of NTDs, but might also decrease the severity of NTDs, as long as supplementation is started before conception.

PS18.83
Population-based detection of structural variants in normal and aberrant genomes

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Structural Variants(SVs) are key elements in evolution and complex diseases. Their detection from high-throughput sequencing(HTS) data has evolved substantially increasing its sensitivity to smaller events and breakpoints resolution. However the fraction of false positive predictions remains non-negligible and few efforts have been directed toward problematic regions such as low-mappability or repeat-enriched genomic regions, known to be rich in SVs but generally excluded from analyses. After revealing important complex technical bias in HTS, we propose to use a large set of experiments and a population-based approach to robustly identify of abnormal regions genome-wide.
Comparing read coverage across hundreds of samples requires an appropriate normalization step. We show that a general normalization is not sufficient to correct systematic sample-specific variation and develop a flexible and targeted approach. The statistical test for each bin uses a Z-test-like score adjusted by a robust multiple-testing correction. We test our approach on more than 100 normal and tumor whole-genome paired datasets from three different cancer resequencing projects. We show that more concordant germline events and tumor-specific ones are detected, compared to other approaches. Very few regions of the genome were excluded and a number of SVs were detected in low-mappability region. The comprehensiveness and extended genomic coverage of the approach will benefit the characterization of variation in low-mappability regions as well as cancer and disease related studies where complex SVs play an important role.

PM18.84
Infering variants contributing to synthetic association

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It has been suggested that association of common variants with complex diseases can be created by multiple rarer variants. While the hypothetical properties of such synthetic associations have been elaborated, so far no method has been provided for the systematic retrieval of genetic variant sets that create a synthetic association. Variants giving rise to synthesis might be located several Mb away from the major association signal. Testing all possible combination of n nearby variants results in an exponential number of tests, 2^n-1. In view of this, the need for an efficient search strategy is obvious. In this work, we define filter criteria based on linkage disequilibrium measures and allele frequencies which reflect expected properties of synthesizing variant sets. From those we obtain potentially synthesizing sets and, by using the identified variants as covariate parameters in a regression model, subsequently evaluate whether they actually explain the main association signal. We apply our algorithm to confirmed consensus susceptibility loci of Alzheimer's disease which have been published recently in a large meta-analysis study.

PS18.85
Influence of Ethnicity in Association of CAPN10, PPARG and PGC-1α Genes with T2D from North-West Indian Population

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Type 2 diabetes (T2D) is a global public health crisis threatening all economies especially those of the developing countries. India exhibits different ethnic population structure comprising of various caste groups which practice strict endogamy. The present study investigated the association of CAPN10 (SNP-43, SNP-19, SNP-63), PPARG (Pro12Ala) and PGC-1α (Thr612Met, Thr528Thr, Gly482Ser, Asp475Asp, Thr394Thr, IVS2+C>A) polymorphisms in 1125 samples including 554 T2D cases and 571 controls from Bania, Brahmin and Jat-Sikh ethnic groups of North-West India. Minor allele of CAPN10 SNP-43, -19 polymorphisms provide risk towards T2D in Brahmin and Jat-Sikh groups; SNP-63 in Bania and Jat-Sikh groups. G-allele of PGC-1α Gly482Ser polymorphism was found to play protective role against T2D in Jat-Sikh group (p=0.002). Intriguingly, it provided 1.48 fold increased risk towards T2D predisposition in Bania group (p=0.009) suggesting population specific risk towards T2D. However, no association of PGC-1α (Asp475Asp) and PPARG (Pro12Ala) polymorphisms were seen in any group. In CAPN10 gene, the 212-haplotype in Bania group, 221-haplotype in Brahmin, 211 and 221-haplotypes in Jat-Sikh group conferred increased risk towards T2D susceptibility. PGC-1α haplotypes GGGGCA, GGGGTC in Brahmin; GAAGTC, GGGGTC in Jat-Sikh group provide 1.5-5 fold increased T2D risk. Differential pattern of genetic association and haplotypes of studied genes emphasized the role of ethnic-heterogeneity in T2D. The inconsistency of genetic effects of studied polymorphisms across populations from different ethnicities was investigated through meta-analysis indicating the role of population stratification. Thus, for risk calculation and proper medical intervention, knowledge of the ethnicity and nature of variation in risk factors needs serious attention.

PM18.86
Large scale meta-analysis of genome-wide data after 1000 genome imputation identifies multiple new loci associated with telomere length.

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Introduction: Mean leukocyte telomere length (LTL) exhibits considerable interindividual variability and has high heritability estimated at 0.70 (95% CI 0.64-0.76). Both shorter and longer LTL have been shown to associate with age-related diseases, including coronary artery disease (CAD) and some cancers. We previously reported seven loci associated with LTL. Using these in a genetic risk score analysis implies a causal link between shorter LTL and increased CAD risk. However, the extent to which the association of shorter LTL with other age-related disorders is causal in nature remains unclear. Identifying additional genetic variants that affect LTL and testing their association with disease through mendelian randomisation approaches could clarify any causal role.
Materials and methods: We report preliminary results from a genome-wide meta-analysis of LTL in up to 44,708 individuals, from 21 cohorts, after imputation using the 1000 genomes reference panel.
Results: We have redefined the lead signal for many of the known loci. Using an FDR of 1% in an additive model we identify ten novel loci affecting LTL. We also performed a recessive analysis which identified an additional 13 loci using an FDR of 5%. The novel loci includes both genes involved in telomere biology (ATM, DCAF4, POT1) as well as genes with no known involvement (MPHOSPH6, DCC).
Conclusions: Imputation to a large reference panel and assessing both additive and recessive modes of inheritance has brought the total number of loci associated with telomere length to 30.
Grants & fellowship: The ENGAGE Consortium was funded by the European Union.

PS18.87
Frequency of COL4A3/COL4A4 mutations amongst families segregating glomerular microscopic hematuria and evidence for activation of the unfolded protein response - Focal and segmental glomerulosclerosis is a frequent development during ageing.

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Familial glomerular hematuria(s) comprise a genetically heterogeneous group of conditions which include Alport Syndrome (AS) and thin basement membrane nephropathy (TBMN). Here we investigated 57 Greek-Cypriot families presenting glomerular microscopic hematuria (GMH), with or without proteinuria or chronic kidney function decline, but excluded classical AS. We specifically searched the COL4A3/A4 genes and identified 8 heterozygous mutations in 16 families (28,1%). Eight non-related families featured the founder mutation COL4A3-p.(G1334E). Renal biopsies from 8 patients showed TBMN and focal segmental glomerulosclerosis (FSGS). Ten patients (11.5%) reached end-stage kidney disease (ESKD) at ages ranging from 37-69-yo (mean 50,1-yo). Next generation sequencing of the patients who progressed to ESKD failed to reveal a second mutation in any of the COL4A3/A4/A5 genes, supporting that true heterozygosity for COL4A3/A4 mutations predisposes to CRF/ESKD. Although this could be viewed as a milder and late-onset form of autosomal dominant AS, we had no evidence of ultrastructural features or extrarenal manifestations that would justify this diagnosis. Functional studies in cultured podocytes transfected with wild type or mutant COL4A3 chains showed retention of mutant collagens and differential activation of the unfolded protein response (UPR) cascade. This signifies the potential role of the UPR cascade in modulating the final phenotype in patients with collagen IV nephropathies.

PM18.88

Recessive inheritance and inbreeding in Thyroid cancer

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Genome-wide association studies (GWASs) have identified several single-nucleotide polymorphisms (SNPs) influencing the risk of thyroid cancer (TC). Most cancer predisposition genes identified through GWASs function in a co-dominant manner, and studies have not found evidence for recessively functioning disease loci in TC. Our study examines whether homozygosity is associated with an increased risk of TC and searches for novel recessively acting disease loci. Data from a previously conducted GWAS were used for the estimation of heritability, the detection of runs of homozygosity (ROH) and the determination of inbreeding to unravel the influence on TC. Association on a SNP-by-SNP basis reached genome-wide significance at a level of P<10-8, with eight SNPs representing true differences in homozygosity between cases and controls. The rate of ROHs per person was significantly higher in cases than in controls. The average ROH size and length of ROHs per person was also higher for cases than controls. A total of 16 recurrent ROHs of rather short length were identified although their association was not significant at a genome-wide level. Several recurrent ROHs harbor genes associated with risk of TC. All of the ROHs showed significant evidence for natural selection (iHS, Fst, Fay-Wu's H). The higher inbreeding among cases support the existence of recessive alleles that cause TC. Although regions of homozygosity were rather small, it might be possible that variants within these ROHs affect susceptibility to TC and may function in a recessive manner.

PS18.89

METAINTER: meta-analysis tool for GWAS allowing for interaction

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Introduction: Meta-analysis of summary statistics from Genome-wide association studies (GWAS) may lead to discovery of new susceptibility loci without exchange of genotype data. Well-known p-value combination methods can be applied to an arbitrary association test. However, these methods do not provide summary effects and are underpowered for high-dimensional models. Results of related genome-wide interaction analyses (GWIA) have rarely been meta-analyzed due to complexities underlying the process of their synthesis.

Materials and Methods: Summary statistics of single-SNP GWAS can be integrated in one-step meta-analysis. For two-SNPs GWIA, we suggest a two-stage procedure to guarantee computational feasibility. At stage I, complete GWIA is conducted in each study participating in meta-analysis using a fast pre-test. All studies retain top ranking results to merge them in a „joint" list of SNP pairs. At stage II, the joint list is analyzed in each study by an advanced (regression) test. The results of stage II can then be meta-analyzed using proper software. We introduce an efficient, powerful and freely available tool METAINTER, which implements recently developed method for the synthesis of multiple regression slopes and three further meta-analysis methods. METAINTER enables meta-analysis of interaction tests, single-marker tests with several degrees of freedom, global haplotype tests etc. We conducted a real data analysis of six GWAS of type 2 Diabetes (T2D). The results were meta-analyzed with METAINTER.

Results: We obtained evidence for interaction between SNPs in PPP2R2C, associated with T2D and close to LOC101928448, associated with body fat distribution.

Conclusions: We introduce an efficient and powerful pipeline for the meta-analysis of GWIA.

PM18.90

Sex specific GWAS reveals new loci associated to red and white wine liking.

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Recent studies in the genetics of wine liking have found a strong association between white wine liking and the HLA-DOA gene. However given the great differences shown in wine preference by man and women we have decided to conduct a sex specific GWAS on red wine and white wine liking using 5 isolated population coming from Italy (INGI-CARL , INGI-FVG , INGI-VB), Netherland (ERF) and Central Asia (SR) using a total of 1682 man and 2184 women. GWAS on man identified 4 genome wide significant loci (p<5x10-8). Best hits for each locus are: rs78560355 (p=7.5x10-10) close to the PLCL1 e rs112080424 (p=3.9x10-8) close to TNS3 for red wine and rs35662810 (p=1.2x10-8) close to LOC100288392 and rs72517376 (p=2.14x10-8) inside LGALS2 for white wine. PLCL1 in particular has been previously associated to the maximum number of alcoholic drinks consumed at one time. We decided to verify if the identified SNPs had an impact on alcohol consumption. We thus created a score based on the discovered SNPs. The value for each person was estimated as: wine_liking_score = genotypeSNP * betaSNP. The created score showed a significant and similar effect in the CARL and FVG populations (lbeta 0.13 and 0.11 respectively, combined p=0.003). The identified genes are one of the first examples of association between genetic variants and common foods. Further more we have shown that the identified genes have an effect on total alcohol consumption suggesting a causal relationship between wine liking and alcohol consumption.

PS18.91

Paternal genetic landscape of Croatian Eastern Adriatic islands in a wider European context

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Croatian Eastern Adriatic islands represent well-characterized genetic isolates, concerning their ethnohistory, biological traits, disease prevalence, migration patterns and environmental and sociocultural characteristics. Such small communities can reflect large demographic processes that happened in human prehistory and history and give us insight into the ancient migratory paths and forming of the Croatian and Southeast European gene pool. The aim of this study was to evaluate the level of isolation of insular populations based on the observed haplogroup distribution and frequencies of 720 samples from 12 Croatian subpopulations and to define the main processes that have shaped present Y chromosome variation of Eastern Adriatic islands and Croatia in general. A high level of haplogroup and haplotype diversity has been detected in the overall sample and the observed haplogroup frequency distributions correspond to an average European population, suggesting a dynamic gene flow in this region throughout history. The PCA plots show that the position of different Croatian subpopulations mostly corresponds to their actual geographic location. Also, it is noticeable that most of them are stretched along an axis, depending of the portion of I2a2-M423 and R1a-M458/M558 sublineages in their sample. Although the

studied insular populations show an expected (mostly Paleolithic) paternal genetic structure corresponding to their geographic location, genetic structure of some islands exhibits interesting results due to the significant effect of evolutionary forces and specific historic episodes (like Lastovo, Cres and Dugi otok).

PS18.93

Phylogeographic refinement of human Y chromosome haplogroup E provides new insights into the early dispersal of herders in sub-Saharan Africa

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Recently, a high number of Y chromosome SNPs has been discovered through next generation sequencing studies, but the geographic distribution for most of these variants remains largely unexplored.

Haplogroup E is the most common human Y chromosome clade within Africa and its internal branches have been linked to a wide range of human movements. To increase the level of resolution of haplogroup E, we disclosed the phylogenetic relationships among 729 mutations found in 33 haplogroup DE Y-chromosomes sequenced at high coverage in previous studies and further dissected the E-M35 subclade by genotyping 62 informative markers in about 5000 samples from 118 worldwide populations.

The phylogeny of haplogroup E showed novel features compared to the previous topology, including a new basal clade. Within haplogroup E-M35, we resolved basal polytomies and assigned all the E-M35* chromosomes to different new monophyletic clades. Through a Bayesian phylogeographic analysis, we associated each node of the tree to specific geographic areas. By this analysis, we identified a new E-M35 sub-Saharan clade, which originated about 11 kya in the northern part of the Horn of Africa. SNP-based dating, phylogenetic structuring and geographic distribution of this clade (and its sub-clades) are consistent with a multi-step dispersal of herders within eastern Africa and its subsequent diffusion to sub-equatorial areas.

Our results provide new insights into the evolutionary hypotheses about the spread of pastoralism in Africa and increase the discriminative power of the E-M35 haplogroup for use in forensic genetics through the identification of new ancestry informative markers.

PM18.94

Spatial variation of the Y-chromosome: The global patterns and correlations with other genetic systems, linguistic and geography

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We developed the "Y-base" database, which includes frequencies of 500 Y-chromosomal haplogroups in 4200 populations worldwide, with total sample size 142,000. 130,000 Y-chromosomes came from 300 published papers and remaining 12,000 are our unpublished data.

Using this dataset we created the world spatial distribution maps of 230 haplogroups. This World Atlas of Y-chromosomal variation was created by GeneGeo software, which we developed for digital map analysis in gene geography. The zones of sharp changes in frequencies were interpreted as genetic boundaries; the main boundary crosses Eurasia and includes not only mountain (Himalayas and Caucasus) but also steppe segments.

The question arises to which degree patterns of Y-chromosomal variation agree with data on other genetic systems. To answer, we characterized all extant ethnic groups speaking Balto-Slavic languages by mitochondrial DNA (N=6,876), Y-chromosome (N=6,079) and genome-wide SNPs (N=296). We found that genetic distances, based on autosomal and Y-chromosomal loci, show a high correlation (0.9) both with each other and with geography but slightly lower correlation (0.7) with the mitochondrial DNA and linguistic affiliation.

The high-throughput sequencing of the Y-chromosome reveals thousands phylogenetically informative SNPs. Population screening for these markers subdivides old haplogroups with subcontinental zones of spread into multiple young haplogroups with restricted areas - thus providing excellent tools for reconstructing population history. This approach allowed us successfully subdivide C2-M217, N1c-M178, and G1-M285 into 35 new subhaplogroups, to create their frequency distribution maps and estimate the SNP and STR mutation rate on the Y-chromosome.

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PS18.95

Corsican genetic heritage: Y-chromosome story

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The scarcity of human remains in Corsica leads to uncertainties of its first peopling. First modern human might have arrived during Paleolithic, as great glaciations had form bridges between Toscana and Corso-Sardinian Island, allowing humans to cross at different time in Pleistocene.

Conflicting results have been published concerning the genetics of the Corsican populations and their relationship with Sardinian, French and Tuscans populations. It appears obvious that Corsican populations display high heterogeneity, exacerbated between North and South; this is confirmed by anthropological, archeological and linguistic data. Moreover, continental historical genetic input could be modest, despite many historical campaigns. Thus, the genetic characteristics of the Corsican population could be the result of Neolithic or Paleolithic migrations.

The objective of the present study is to get an insight of the Corsica peopling by the analysis of the Y-chromosome. This study comprises data from populations from the Mediterranean zone, totaling 890 individuals. Samples were collected from 18 populations from Corsica, 1 from Provence and 3 from Toscana.

All samples were type for the 19 main haplogroups and according to each sample genotype, further analyses were performed: 84 binary markers were genotyped and 53 were informative.

Haplogroups profile in Corsica showed some signature from both France and Toscana, but mostly display its own specificity. The strongest genetic structure was encountered when the populations were considered into four main geographical groups: northwest, south and east, northern peninsula, and western region. Because of the haplogroups described here and their diversity, these results support Neolithic and Paleolithic migrations rather than historical movements to Corsica.

PM18.96

Characterization of Beta-thalassemia Mutations in Saudi Patients

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Beta thalassemia is a monogenic disorder characterized by reduced or absent β-globin chain synthesis. More than 200 disease causing mutations have been identified in the beta globin gene; such mutations can seriously affect the production of β-globin chains and consequently cause β- thalassemia.

In some regions of Saudi Arabia such as AlHassa; β- thalassemia occurs at a frequency as high as 3.4% in premarital couples. Meanwhile, no comprehensive mutational screening for the β-globin gene has been carried out so far in Saudi Arabia. This study reports the complete molecular screening of the entire β- globin gene in 120 Saudi β- thalassemia patients. Sequencing of the entire open reading frame and LCR of the β-globin gene identified 7 homozygous disease causing point mutations and 9 compound heterozygous disease-causing mutations of these one is novel. MLPA analysis revealed a number of deletions in our patients. In addition haplotype analysis using 5 restriction enzymes identified 22 different haplotypes in our cohort of patients. Carrier status was also determined in enrolled family members. This study characterizes the common mutations and variants in the β-globin gene in Saudi Arabia and reports new variants and mutation.

This study was supported by grants from King Abdul-Aziz City for Science and Technology (KACST), King Faisal specialist Hospital and research center (KFSH&RC), and Kind Saud University (KSU).

PS19.01

An integrated approach to the introduction of Genomics into the NHS in England

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As part of the NHS contribution to the 100,000 Genomes Project, NHS England committed to putting in place arrangements to enable the acquisition of samples and accompanying clinical data of sufficient quality to enable whole genome sequencing under the contractual arrangements between Genomics England (a company owned by the Department of Health set up to deliver the 100,000 Genomes Project) and Illumina (the company Genomics England procured to deliver the sequencing and support the analysis of the resulting genome).
Since July 2014, a transparent and rigorous procurement exercise has been conducted to commission and designate the first ever Genomic Medicine Centres (NHS GMCs). The service specification detailed every element of the sample and data pipeline in participants with rare disease and cancer (from acquisition to validation of whole genome sequence findings to feedback to clinical teams and participants) and provides not just the contractual basis for service delivery but a baseline for measuring improvement and the quality of outcomes. This will serve as a catalyst to a broader transformation programme to introduce genomic technologies into clinical care across the NHS in England in a systematic and sustainable way for public and patient benefit, to support scientific endeavour and diagnostic delivery and industry collaborations.
Already we have seen the NHS GMCs adopt innovative patient and public co-production to design their genomics service, the creation of genomics MDTs spanning a multitude of specialisms, creative workforce training and forward thinking approaches to informatics.

PM19.02

Perspectives of adolescents regarding their genetic counselling experience: a qualitative study

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Introduction: Adolescence is an intense period of development that involves creating a sense of identity, autonomy, relationships and values. This can be complicated by having a genetic condition. Genetic counselling can play an important role in providing information and support to this patient population; however, resources and guidelines are currently limited. We sought to investigate the experiences and perspectives of adolescents with a genetic condition regarding their genetic counselling interactions.
Methods: This study utilized a qualitative exploratory approach, specifically the Interpretive Description methodology. Eleven semi-structured interviews were conducted with adolescents diagnosed with a genetic condition who received genetic counselling between the ages of 12 and 18 years at The Hospital for Sick Children. Transcripts were analysed thematically using qualitative content analysis.
Results: Three major interrelated themes emerged from analysis: (1) understanding the genetic counsellor's role; (2) increasing perceived personal control; and (3) adolescent-specific factors influencing adaptation to one's condition. Additionally, a list of suggested tools and strategies for genetic counselling practice were elucidated.
Conclusions: To our knowledge, this study is the first to explore the perspectives of adolescents with respect to their genetic counselling experience. Information gleaned from this study will contribute to the development of an adolescent-focused framework to enhance emerging genetic counseling approaches for this patient population, and will facilitate the transition process from pediatric to adult care within patient and family-centered contexts.
This study was funded by The Hospital for Sick Children Trainee Start-Up Fund.

PS19.03

Multidisciplinary counselling in genetic tests for Amyotrophic Lateral Sclerosis

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Amyotrophic Lateral Sclerosis (ALS) is a devastating rare late onset neurodegenerative disease which affects motor neurons in the primary motor cortex, brainstem and spinal cord.
The etiopathogenesis of the disease is still known however several genetics factors have been identified and the hypothesis of multifactoriality is the more accreditated.
The discovery of genetic abnormalities causing familial (FALS) and sporadic (SALS) ALS allowed patients to access to genetic test; consequently, the elaboration of a clinical, genetic and psychological asset allowing to cope genetic issues is required. To date, no specific guidelines for neurological disorders genetically inherited exist, with the exception of Huntington's disease (HD).
In 2013 our team group introduce in the context of genetic counselling, a multidisciplinary approach characterized by the integrative participation of three different professionals, psychologist, geneticist, and neurologist in order to face all aspects related to the pathology and to understand and manage information in a personal and familial way.
We report here the clinical experience of a project dedicated to the development of a specific model of genetic counselling for ALS patients, based on suggestions on genetic counselling for this disease.

PM19.04

Reflections on the reactions of breast cancer patients who do not fulfill the criteria for BRCA1 and BRCA2 genetic testing

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Since 2004, cancer patients have been referred to the Cancer Genetics Clinic (CGC) for tailored hereditary risk assessment and genetic counselling based on the type of cancer, family history and age of diagnosis. For breast cancer patients, the CGC team has recently started to use the Manchester scoring system (MSS) in order to assess patients' hereditary risk and eligibility for BRCA1 and BRCA2 genetic testing. The MSS is a relatively new scoring system that is devised to estimate the possibility of identifying pathogenic mutations in the BRCA1 and BRCA2 genes, with a 10% cutoff level for testing eligibility. Patients who score less than 10% on the MSS following risk assessment are not eligible for genetic testing. These patients are offered a careful explanation of the eligibility criteria, risk assessment, psychosocial counselling and are advised on the importance of their continuation with cancer screening. They are also advised to contact the CGC in the event that they or a family member has a new cancer diagnosis so that an updated risk assessment is offered to them. In this poster, we will reflect on the impact of using MSS at CGC as well as comment on the various reactions of these patients who are not offered genetic testing because they scored below the cutoff level.

PS19.05

Personalized medicine in cancer prevention- BRCA screening in unaffected Ashkenazi Jewish women. Randomized controlled trial of different pre-test strategies

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BRCA1/2 carriers have high breast/ovarian cancer risks that can be diminished by risk-reduction surgeries. However, approximately half of carriers lack significant family history, and would only be identified through general testing. The Ashkenazi Jewish population is a model for such screening, given high prevalence (1/40) and testing sensitivity (>95%) of three common mutations. Large scale testing may require adjusting the counseling process. We are examining the impact of excluding pre-test face-to-face genetic counseling (GC) in the population screening setting.
Ashkenazi Jewish women age ≥ 25 years, were randomized to two pre-test arms: traditional GC vs. written information only (WI). Feelings, knowledge and attitudes toward testing were assessed using questionnaires one week (Q1) and 6 months (Q2, ongoing) post-testing.
Among the first 309 participants we identified six carriers (1.9%) Another 6 carriers were identified by cascade testing in 10 relatives of these carriers. At Q1, satisfaction was very high or high in both GC (62%, 33% respectively) and WI (54%, 41%), but higher in GC (p=.002). IES (impact of events)

scores were similar in both groups. At Q1, personal perceived control (PPC) scores and knowledge were higher in GC (p=.005; p=.0002), but absolute differences were small; PPC: 0.17 of 2 points, knowledge: 1.14 of 10 points. At Q2, only PPC scores remained higher in GC: 1.46 vs. 1.23 in WI (p=.008). These ongoing results suggest that in the screening setting, forgoing pretest GC may be a legitimate alternative. This option could be considered in cases of logistic or cost limitations.

PM19.06

Evaluation of an information day for individuals with BRCA1 and 2 gene mutations in the East Anglian region (UK): participant feedback and reflections

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Background: Our aim was to address ongoing support and management needs of families with hereditary cancer syndromes. With ever increasing pressure on genetics services, a cost- and time- effective approach that has been widely adopted to address this is to organise Information Days. These provide families/individuals with up to date information about medical management and psychological implications. Following our recent information day, we present our participants' feedback and our reflections for future programme development.
Programme: All 561 individuals with a known pathogenic mutation in BRCA1/2 who underwent genetic counselling and testing through the East Anglian Medical Genetics Service were invited to attend the day, along with family/friends. 196 people attended the day. The programme included presentations about BRCA mutations, risk reducing surgery, the support group, FORCE, and research, as well as a 'Q&A' panel discussion. 59 feedback forms were completed, many of which reflected the views of the family rather than of individuals.
Feedback and Reflections: The feedback from participants was overwhelmingly positive, with many expressing how valuable they had found the day and requesting future events. In particular they commented on their increased knowledge and understanding, and the benefits of a forum to meet with other BRCA1/2 families. Several requested that future events better address the support needs of men and have a stronger focus on addressing psychosocial aspects. The feedback will direct how we propose to develop an ongoing programme in our catchment area.

PS19.07

Recommendations vs. Reality: Genetics referrals for all invasive serous ovarian cancers

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It is well recognized that having a diagnosis of invasive serous ovarian cancer (SOC) is associated with an increased risk of having a BRCA1 or BRCA2 mutation. Therefore, numerous guidelines recommend that women with this diagnosis are offered genetic testing irrespective of family history or age at diagnosis. Despite such guidelines, previous data from the Princess Margaret Cancer Center (PM) in Toronto, Canada demonstrate that only 23% of women diagnosed with SOC were seen for genetic counselling. To improve genetic counselling uptake, changes in pathology reporting, appointment scheduling, and referring physician education were implemented. Following these interventions, referral and genetic counselling uptake rates were examined for all SOC cases at PM between 2010 and 2013. Of 430 SOC cases, 59% (253/430) were referred for genetic counselling and 54% (233/430) attended an appointment. Of those who received genetic counseling, 94% (220/233) proceeded with genetic testing and 23% (50/220) harboured a BRCA1 or BRCA2 mutation. Notably, 34% (17/50) of women with a mutation had no family history of breast or ovarian cancer. Despite changes to increase uptake of genetic counselling, a significant number of eligible women are still not receiving genetic testing. Considering the high mutation rate in this population and the value of such results in determining treatment and identifying high-risk families, it is imperative that all women diagnosed with SOC are offered genetic testing, irrespective of family history or age at diagnosis.

PM19.08

How reassuring is a negative result? Rapid genetic testing of BRCA1 and BRCA2 during treatment for breast cancer.

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Women diagnosed with breast cancer, who also have a BRCA1 or BRCA2 mutation have a high risk of a second primary breast cancer (1). Offering these patients the opportunity to find out their BRCA status between diagnosis and surgery could help inform their surgical treatment and also give them the opportunity to consider including a prophylactic element to their surgery.
From the beginning of 2014 breast surgeons in Edinburgh have been able to refer patients, who meet a certain threshold, for rapid testing of BRCA1 and BRCA2. The aim being to get results back to patients and their surgeons in time for them to be of use in making surgical decisions.
In this poster we will present the criteria that were used to target the testing, and how the referral process was managed. We will present the outcome of the testing in this small group of patients and also asses the influence the result had on surgical decisions by comparing the patients stated intentions about surgery prior to testing and their eventual surgical treatment.
We will conclude with some brief reflections on the counseling and ethical issues genetic testing during diagnosis raises.
1. Malone KE, et al. Population-based study of the risk of second primary contralateral breast cancer associated with carrying a mutation in BRCA1 or BRCA2. J Clin Oncol. 2010;28:2404-10.

PS19.09

Should women with breast cancer before 40 years be included in rutine genetic test in the Genetic Counseling Units? The Castilla y León experience

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Breast cancer (BC) is the most common invasive cancer worldwide in women and it is also the leading cause of death from cancer amongst women. BC in young women is rare and only 5-7% occurs in women under 40 years.
Nearly 5-10% of patients suffering from BC have genetic predisposition. Most of hereditary breast and ovarian cancer syndromes are caused by a germline mutation in the BRCA genes. Genetic counseling allows to find subjects with hereditary genetic predisposition to cancer, to implement preventive measures reducing the incidence of these tumors and early diagnosis to improve survival.
The aim of this study was to review the inclusion criteria of women with BC below 40. From the unit of genetic counseling of Castilla y Leon between 2003-2013, 206 women diagnosed with BC under 40 were included in this study. Genomic DNA was isolated from peripheral blood and BRCA1/2 coding regions and intron-exon boundaries were amplified by PCR and analyzed by Sanger sequencing.
The prevalence of a BRCA1/2 germline pathogenic mutation was 22 (10,7%). From these patients 18,1% did not have familial history of breast/ovarian cancer. 72% of these mutations were found in BRCA1 where 75% of these carriers have familial history of breast/ovarian cancer. In relation to 184 non-carriers patients, 81 women had positive familial history.
Our results suggest that women below 40 without familial history should not be included for BRCA1/2 genetic studies although it is necessary to perform an economical analysis before take the definitive decision.
Supported by PI13/01741 and Consejería de Sanidad de la Junta de Castilla y León

PM19.10

Difference and similitude regarding breast and ovarian screening program and prophylactic surgery guidelines in women carriers of a BRCA mutation: results of a European online survey

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Introduction: Female carriers of a *BRCA* mutation have a high risk of developping breast and/or ovarian cancer. In France, the National Institute of Cancer published in 2009 guidelines for breast and ovarian cancer early detection and prevention in female carriers. As a part of a reflexion dealing with these guidelines, we initiated a European study on breast screening program and gynaecological monitoring aimed at female carriers of a *BRCA* mutation.
Material and Methods: we collected information regarding these programs through an online survey. Health professionals (physicians, medical gene-

ticist, nurses, oncologists, gynecologist, psychologists, genetic counsellors) in charge of *BRCA* mutation carriers in the context of a genetic clinic were asked to participate to this survey through the ENGNC group electronic mailing list.
Questionnaire regarding *BRCA* mutation carriers took into account three different situations: 1) women non-affected with cancer, 2) women affected with breast cancer, 3) women diagnosed with ovarian cancer. For each of them, participants were asked about breast-awareness, clinical breast examination, breast and ovarian screening program, prophylactic surgery (mastectomy and/or salpingo-oophorectomy) and chemoprevention programs.
Results : We already obtained questionnaires replies from nine European countries: France, Netherlands, Ireland, England, Hungary, Germany, Italy, Sweden, Switzerland. A second round of letters was sent in order to encourage additional participants to complete the survey.
We will report about similitude and differences in screening and prophylactic surgery guidelines according to each group of patients, and their country or evenly provinces of residence.

PS19.11
Inherited childhood cancers: parents' perceptions of children's information needs

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Multiple Endocrine Neoplasia type 1 (MEN1) and type 2 (MEN2) are autosomal dominant cancer syndromes that are associated with significant morbidity and mortality. Early genetic testing and diagnosis of MEN1 and MEN2 can allow for medical care to improve cancer related outcomes. There is little research on communication with children about genetic testing for childhood cancers. This study explored parents' experiences and perceptions of children's information needs for genetic testing for MEN1 and MEN2. Semi-structured qualitative interviews were conducted with seventeen parents whose children had been tested for either of these cancer syndromes in the twelve months preceding the time of recruitment. The findings show that parents found it difficult to broach the subject of predictive genetic testing for cancer with their children, and many would have valued support to do so from healthcare professionals (HCPs). Parents also believed their child was over-burdened with information from the HCPs, and would have valued a pre-consultation meeting with the HCP only to ensure the information was tailored to the child's needs and temperament. Parents had differing views about the use of the word 'cancer' in consultations with their child. While some believed it should be avoided because of its perceived association with imminent death, others explained that the familial nature of the condition meant that their child could associate cancer with living a 'normal' life. These findings have implications for the way in which HCPs communicate with parents and children about genetic testing for MEN1 and MEN2 to improve efficacy of consultations.

PM19.12
Cancer genetics management for primary care providers

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The American Society of Human Genetics and The Jackson Laboratory have developed and implemented an interactive educational program to improve the clinical skills of primary care providers (PCPs) in assessing and managing genetic risk for cancer. This blended learning program consists of 6.5 hours of in-person content, monthly communications, and access to online resources. The program addresses learning objectives that help providers learn about and practice: collecting a targeted family history; identifying red flags and patterns of inheritance; assessing the clinical utility of testing; facilitating patient decision-making; communicating the benefits and limitations of testing; and using guidelines to manage care. The program was piloted with 21 PCPs in the U.S. in November 2014. Participants completed knowledge, attitudes, and confidence assessments pre and post, which were analyzed by paired t-tests. Participants demonstrated improved confidence in their ability to assess hereditary cancer risk, determine appropriate referrals, and discuss the benefits, risks, and limitations of genetic testing with patients (all $p<0.001$), increased agreement with attitudes related to genomic risk assessment ($p<0.05$), and improved knowledge related to risk assessment, genetic testing, and clinical management ($p = 0.01$). These early results suggest that this innovative program can build PCP skills in the assessment and management of hereditary cancer, supporting improved patient care. Based on this successful pilot, the program will be implemented more broadly and will serve as a framework for future programs and expanded audiences to improve clinical integration of genetics into practice.

PS19.13
Population genetic carrier screening for cystic fibrosis, fragile X syndrome and spinal muscular atrophy: Exploring experiences of carriers identified through the VCGS Reproductive Genetic Carrier Screening program

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Due to advancing genetic technologies, population carrier screening for multiple inherited conditions can now be offered. This research aimed to explore how women experience undergoing carrier screening for three common inherited conditions; cystic fibrosis (CF), spinal muscular atrophy (SMA) and fragile X syndrome (FXS), through the Victorian Clinical Genetics Services (VCGS) Reproductive Genetic Carrier Screening (RGCS) program.
This study adopted a qualitative approach based on phenomenology. Ten female participants were recruited and in-depth semi-structured interviews undertaken. Two received a pre-mutation carrier result for FXS, three received a carrier result for CF and five for SMA. Coded transcripts were analysed using thematic analysis to identify emerging themes.
The majority of participants were pregnant during screening and described the decision to have the test as straight forward. Participants' experienced emotional responses (anxiety and stress) whilst waiting for their partner's test result and completed online research to discover more about the relevant condition during this time. Participants were in favour of population carrier screening, preferably offered prior to conception.
The findings of this study elucidated that genetic counsellors (GCs) play an essential role within this program, supporting couples after they receive a carrier result given the varying consent processes undertaken prior to screening. The implementation of Internet resources and GC facilitated guidance to access reliable online information is crucial to help empower couples and assist the coping process. Improving awareness of the availability of population carrier screening within the community will also help improve knowledge levels and facilitate preconception screening.

PM19.14
Barriers and facilitators of cascade screening for hereditary breast/ovarian cancer in a statewide randomly selected sample

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Genetic counseling is recommended for young women diagnosed with breast cancer and their close relatives. Cascade screening is a new public health intervention that promotes systematic familial testing among relatives of individuals with known mutations. In an effort to assess the feasibility of statewide cascade screening, we examined the willingness of young breast cancer survivors (YBCS) invite their high-risk relatives to participate in such a program.
A randomized trial aiming to increase awareness of cancer genetics, identified a random sample of 3000 YBCS, diagnosed 25-45 years old, and stratified by race (Black/Other), through the Michigan Cancer Surveillance Program. YBCS' responses helped identify high-risk, unaffected, female relatives. A linear regression analysis, guided by the Theory of Planned Behavior, examined demographic and clinical characteristics, access and attitudes towards healthcare services, family history, and family environment as predictors of the proportion of eligible relatives YBCS were willing to contact (number willing to contact/ number of all high-risk relatives).
Responses from 859 eligible YBCS (33%) helped identify 1842 high-risk relatives; YBCS were willing to contact 1271 (69%). Willingness to contact a greater number of relatives ($\beta=23.2$), positive family history of cancer ($\beta=1.0$), and higher cancer self-efficacy ($\beta=0.9$) were associated with contacting a greater proportion of relatives. Having a larger family ($\beta=-15.8$) and being Black ($\beta=-.9$) were negatively associated with the proportion of relatives contacted.
Most YBCS were willing to contact relatives for cascade screening, suppor-

ting the feasibility of this approach. Results suggest barriers and facilitators in disseminating information about hereditary breast/ovarian cancer among families.

PS19.15

consanguinity and recessive disorders in East Lancashire (UK)- A multi-disciplinary approach

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Populations practicing consanguinity are at increased risk of having children with autosomal recessive genetic disorders. In Blackburn with Darwen (BwD), families of South East Asian heritage account for 25% of the population and the majority are married to a relative. The infant mortality rate in BwD is the highest in the UK (HSCIC 2014), with the increase largely attributed to autosomal recessive disorders.

An Enhanced Genetic Service was developed in BwD in 2012, to provide genetic services to affected ethnic minority families. Whilst contributing significantly to the genetic management of these families, lack of awareness of genetic issues in the community was apparent; amongst health care practitioners as well as the general public. It was clear that there were also barriers to accessing genetic services for families where culture, language and lack of information reduced opportunities for obtaining appropriate support.

The current, genetic counsellor led initiative embraces a multi-tiered approach, aiming to improve knowledge of genetic issues affecting the ethnic minority population in the community, to encourage engagement with genetic services and to co-ordinate a package of medical and social support.

The unique element of this innovation project has been the establishment of Genetic Outreach workers, linking in to the genetics service, and the strong working partnership that has been developed with community based health care services and voluntary sector agencies.

The development and outcomes of this project will be presented. These have implications for other areas within the UK and Europe that have similar population demographics.

PM19.16

Developing a Master of Science in genetic and genomic counselling in Scotland a collaboration between the West of Scotland Genetic Services and the University of Glasgow

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Members of the West of Scotland genetics service contribute to the University of Glasgow's Master of Science (MSc) in medical genetics. The course was established in 1984 and runs annually for 30 students. In 2014 it was awarded the United Kingdom (UK) Prospects Postgraduate Award for best Postgraduate Teaching Team.

Each year undergraduate and post graduate students ask about becoming genetic counsellors in Scotland. Currently in the UK there are two MSc courses in genetic counselling one based at Manchester University in England and the other at the University of Cardiff in Wales. Both these courses are accredited by the Genetic Counsellor Registration Board (GCRB) and the European Board of Medical Genetics (EBMG). For Scottish students wishing to become registered genetic counsellors in the UK this means applying to courses in Manchester and Cardiff.

Genetic counsellors in Scotland are now working with Glasgow University to develop a MSc in genetic and genomic counselling which will run alongside the medical genetics course. This new course will have to meet Masters level for university approval and it will have to meet the EBMG and GCRB criteria for accreditation. The team have sought opinions from experts in the fields of genetic counselling and education which have proved invaluable highlighting areas for improvement to ensure the MSc in genetic and genomic counselling course meets the required standards.

The course will be based at the university's new education centre at South Glasgow University Hospitals. The course is seeking accreditation prior to enrolling students.

PS19.17

Genetic counselling services in Germany: outcomes of the GenBIn-Study

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Until recently, nationwide empirical data on the structure and utilization of genetic counselling services were not available for Germany. This lack impacts an informed assessment of the development of services after the enactment of the German Genetic Diagnostics Act (GenDG). The GenDG regulates the use of genetic information and materials in the course of genetic testing. All genetic testing is subject to indication and prescription by a licensed physician, requesting binding qualified counselling and information of patients. A Gene Diagnostics Commission (GEKO) has been tasked to issue guidelines related to each individual paragraph of the act. Guidelines for the provision of genetic counselling services were issued in 2012. Objectives: to create a databank that allows for comparing the status quo ante in 2011 with developments of genetic counselling service structures and utilization after the implementation of the GEKO guidelines. Methods: A retrospective empirical study to describe genetic counselling services in 2011 (PartI), followed by a documentation of services in 2015-2016 (Part II). Results Part I: 33 university-based counselling centres and 78 private practice centres were asked to participate. 2092 counselling cases were obtained. Marked differences were found between university-based centres and private practice centres in regard to: referrers, indication for counselling; reported workload; waiting time and case management. The data will serve as a baseline for data to be obtained in 2015-2016 and will facilitate informed assessment of the impact of the GEKO guidelines. (German Federal Ministry of Health: IIa5-2513-FSB-203)

PM19.18

Challenges of genetic counselling, genetic testing and clinical management of asymptomatic children of parents with catecholaminergic polymorphic ventricular tachycardia (CPVT1)

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Catecholaminergic polymorphic ventricular tachycardia (CPVT1) is a cardiac channelopathy inherited in an autosomal dominant pattern. Characteristically patients present with recurrent fainting, seizures or sudden death after physical activity or emotional incidents resulting from high adrenaline levels. On average symptoms present before the age of 10 years. Genetic testing of RYR2 associated with CPVT1 is readily available. If a parent has a clinical diagnosis of CPVT1 and carries an RYR2 mutation predictive genetic testing (cascade screening) is offered to first degree relatives including children. Genetic testing of an asymptomatic child for a familial RYR2 mutation is a complicated decision and can cause psychological stress for the family. Using case studies we present the genetic counselling issues needed to be explored in order for fully informed consent to be reached prior to predictive genetic testing for CPVT1. These include:

Variability of presentation and incomplete penetrance of CPVT1

Evidence of the pathogenicity of the RYR2 mutation

Difficulties in clinically diagnosing CPVT1 in young children

Exercise restriction (RYR2 mutation carriers to avoid highly competitive sports)

Consideration of prophylactic beta blockade or defibrillators in asymptomatic RYR2 mutation carriers

Emotional impact of testing on the family

Difficulties in quantifying individual sudden death risks associated with CPVT1

Given the nature of these discussions there is a need for close collaboration between clinical geneticists, genetic counsellors, laboratory staff, paediatric and adult cardiologists to enable consistent yet individualised care for the family.

PS19.19

Focus on the public health implications and predictors of carrier testing in cystic fibrosis families.

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Objective: Carrier testing enables relatives of cystic fibrosis (CF) patients to know their status regarding the CFTR mutation that segregates within their family. This test allows prevention in families and thus detection of new 1/4 risk couples. This study assessed the impact of carrier testing and searched for the predictors of uptake to testing.
Methods: Forty patients born in Finistère (Brittany, France) between 1980 and 2004 were enrolled. Family trees were faced with the CFTR gene studies performed in the regional laboratories.
Results: Among the 462 non-parent relatives eligible for testing, 185 had testing, corresponding to an adjusted percentage of uptake of 40.4% (95% CI: [33.8%; 46.9%]). Carrier testing enables detection of 5 new 1/4 risk couples, leading to 8 prenatal diagnoses and 5 terminations. Testing also allows to reassure many relatives (as non-carrier by testing or deduction, n=247), plus 60 couples (in whom mates of carrier relatives were negative). Finally, logistic regression adjusted for clustering revealed that the main predictors of being tested were female gender (OR=1.6, p=0.007), having a high a priori risk (OR1/2 vs 1/4=3.5, p=0.006; OR2/3 vs 1/4=31.5, p<0.001) and being over 18 y. at time of diagnosis (OR=2.5, p=0.008). Over 1/3 of the tested relatives had parental project or ongoing pregnancy.
Conclusion: Our study assesses, for the first time in Europe, uptake of CF carrier testing, a critical tool to reassure non carriers and to detect new at-risk couples. It also highlights factors influencing the choice to be tested.
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PM19.20

Meeting the needs of cystic fibrosis carrier parents

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Introduction: Cystic fibrosis (CF) screening for all newborns has been available in Wales since 1996. This identifies a few unaffected carriers in addition to affected children.
Methods: Notes from the newborn screening (NBS) laboratory and the medical genetics service were used to identify children with positive screening tests and those whose families had accessed the medical genetics service. Parents of children identified as CF carriers through NBS between 1996 and 2001 were invited to participate in this study if they had disclosed their child's carrier status. Six families were interviewed about their experiences of finding out their child was a CF carrier and communicating this to their child and wider family.
Results: Parents emphasised the need for a short period between positive screening result and the sweat test distinguishing those affected from carriers. Although some parents had worried about disclosing carrier status to their child, this caused no lasting distress. Parents would have liked to receive advice about discussing carrier status with their children but little was available. No family knew how to seek a genetics referral. Some parents were unclear about the risks of their other children being carriers. Uptake of genetic services by the parents' sibs was lower than in families with an affected child: their wider families often appeared uninterested when parents discussed carrier status.
Conclusion: Professional support for effective communication around the time of a positive screening test is important. Parents may also appreciate support in communicating the implications of carrier status to their children.

PS19.21

Equipping physicians for direct-to-consumer genetic test discussions: a pilot study.

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Direct-to-consumer (DTC) genetic tests are available online, however there is little practical guidance for healthcare professionals (HCPs) and consumers concerning their use. One US study concluded that HCPs should be given "the information and tools they need to help patients make informed decisions about DTC genetic testing". A decision support tool was developed to guide HCPs and patients through a pathway that includes relevant actions and information on the appropriateness of the test. This tool is freely accessible online (http://www.eurogentest.org/index.php?id=939&no_cache=1).
Before the dissemination of any clinical tool, it is essential to conduct an evaluation with a sample of the target population. This small pilot study was conducted to evaluate the decision support tool. Fourth and fifth year medical students were recruited at Plymouth University to take part in an extensive mock consultation with a patient regarding their possible use of a DTC genetic test. In this way we aimed to investigate how useful the tool was in a clinical situation. Participants completed a qualitative debrief immediately following the consultation and their responses were analysed for common themes. It was found that HCPs should be encouraged to familiarise themselves with the tool ahead of an appointment to avoid interrupting the flow of the consultation. The tool was reported to be a useful device for guiding decisions and providing information to patients. With the launch of 23andMe susceptibility testing in the European market, this tool is a timely addition to the primary physician's toolkit.

PM19.22

Down syndrome integrated screening: two-years experience of Public Heath Service in Navarra region of Spain

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Down syndrome (DS) is one of the most frequent causes of fetal pathology, accounting for 8% of all cases of congenital disorders. Its incidence correlates with maternal age and screening programs are recommended.
While not-invasive prenatal diagnosis is rapidly growing as accurate screening method, the costs, the need of confirmation through an invasive test and the lack of long-term experience do not still allow its integration in population-based screening from Public health services. Here we present the experience with DS integrated screening program in Navarra, Spain, provided by the "Servicio Navarro de Salud-Osasunbidea". In more than 10.000 consecutive pregnancies, corresponding to 90% of all pregnancies in the population of reference, which underwent DS screening program, the integration of biochemical markers (PAPP-A, total B-HCG and aFP), sonographic measure of nuchal translucence at week 12 and maternal age allowed a detection rate of Down Syndrome of 88% with a false-positive rate of 3% for a fixed risk of 1:360.
Comparing to previous years in which amniocentesis was offered to all women with positive results on second trimester biochemical studies or advanced maternal age (>35 yearsor more), this DS screening program reduced the number of unnecessary invasive tests by almost 75%, with a higher detection rate. We demonstrate that the integrated screening program for DS in our public health service is effective and a viable option for population-based Down screening programs.

PS19.23

Ethical and legal issue of early prenatal screening in Moscow region

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Analysis of legal regulation and ethics in early prenatal screening in the Moscow region was made. Early prenatal screening program was carried out as part of the Russian national project „Health" and was regulated by the Federal and Regional Governments and the orders of the Ministry of Health. Early prenatal screening program in the Moscow region was started in 2011; more than 150 000 pregnant women (about 70% of all pregnant women living in the region) were examined in 2011-2013. 2380 pregnant women were included in the risk group of fetal chromosomal pathology, invasive diagnosis was carried out in 1668 of them (70%), 416 rejected it. These data may indicate that women used their right to refuse testing, but may be the result of low efficiency of genetic counseling, especially its information components. Analysis of the reasons for rejection of invasive diagnosis was carried out. Frequent reasons were fear of complications (35%), unwillingness to make a decision in early pregnancy (22%), the desire to have a child regardless of his health (19%), confidence that the baby will be healthy (13%). Respondents named medical and domestic reasons for rejection of invasive diagnosis rather than religious or moral principles. The high proportion of women who were afraid of complications and was not ready to make a decision may reflect defects in information and psychological components of genetic counseling for early prenatal screening. This work was supported by the Russian Foundation for Basic Research, project 13-06-00710a

PM19.24

Use of electronic databases and pedigrees in cancer genetic counselling - the counsellee's experience

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Introduction: Individuals and families generally seek cancer genetic counselling because of strong family history of cancer. In Iceland, pedigrees are made using a large genealogy database dating back to at least 1840 and the electronic Cancer Registry holding accurate breast cancer information from 1911 and other cancers from 1954. The aim of this study was to ascertain the acceptability of using databases to make pedigrees and inform on genetic risk assessment.
Material and Methods: Using qualitative methods, counsellees from known BRCA families (n=225) were invited to participate in focus groups via an online bulletin board. A total of 19 participants were allocated randomly to four groups, three of women and one of men only. Data were analyzed using thematic analysis.
Emerging themes arising from the results were motivation for testing, requirements for testing, impact of testing, emotional response and electronic database issues. Participants did not oppose and most trusted the information from the electronic databases. Some addressed concern about data privacy Participants had no specific concerns over other family members' attitudes to use information from databases.
Conclusions: This study has shown that use of databases to confirm genetic information and obtain accurate genealogical information for risk assessment is acceptable to families in Iceland. However, Iceland has a stable population and a long history of using genealogical records and these results may not be transferable to other cultures. Further research is indicated to determine if other populations share similar trust in databases.

PS19.25

Attitudes toward genetic research on children in Japan

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[Aim] Many birth cohort studies have been conducted worldwide. The ethics of genomic research on healthy children is paid attention. The aim of this study is to determine the attitudes of the general Japanese public toward genetic research on children and to clarify factors related to such attitudes based on nationwide surveys conducted in 2013. [Methods] From the general Japanese population, 2,000 people were selected using a stratified two-phase sampling method. In a mail survey administered in 2013, the participants were surveyed regarding the following topics: (1) their attitudes toward genetic testing, the genetic testing of children, and obtaining blood donations from children for research; (2) their level of scientific literacy regarding genomics; and (3)their demographic information and socioeconomic status. [Results] The response rate was 57.7%(1,154/2,000). Conducting genetic testing on children for disease susceptibilities was favored by 58.4% of participants. Regarding obtaining blood donations from children, 46.7% approved, 21.6% disapproved, and 31.7% were undecided. The multiple logistic analysis odds ratio regarding genomic literacy was 1.28 (95% confidence interval = 1.05-1.50). Regarding whether seeking consent from children is appropriate, 45.4% of people answered that seeking consent from a child is acceptable if the child understands the details of the research and 39.2% answered that seeking consent from a child is acceptable if the child can judge the pros and cons of participating in the research. These responses were associated with the level of genomic literacy. [Discussion] The results of this study suggest that people's genomic literacy is essentially related to people's perspective on genomic research on children.

PM19.26

The Italian External Quality Assessment in classical cytogenetics cohordinated by the National Centre for Rare Diseases-ISS: results of the Xth round-2014

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The Italian External Quality Assessment in classical cytogenetics, coordinated by the National Centre for Rare Diseases- Istituto Superiore di Sanità, is an institutional activity; it covers prenatal, postnatal and oncological diagnosis.
All schemes are retrospective; assessment takes into account technical performance, analysis, interpretation and reporting of two cases sent by participants according to SIGU "Guidelines for cytogenetic diagnosis 2013" and ECA "Guidelines and Quality assurance for acquired cytogenetics". In 2013 the category of poor performace was defined.
At the end of the round participants receive a report; until now ten EQA rounds have been completed.
The total number of participants in 2014 was 75; 52, 64 and 28 laboratories participated in the prenatal, postnatal and oncological scheme respectively.
A satisfactory performance was given to the 88% and 75% of participants in constitutional and oncological diagnosis respectively. In constitutional diagnosis eight laboratories were poor performers; one laboratory was poor performer in both prenatal and postnatal diagnosis.
Critical errors leading to poor performances were: a) misleading ISCN errors (four reports); b) misleading ISCN errors and incorrect description of the result or interpretation (two reports); c) incorrect karyotype reconstructions (one case); d) inadequate banding quality (two cases); e) incorrect cytogenetic diagnosis (one case).
In oncological diagnosis seven laboratories were poor performers for: a) incomplete or inadequate analysis (three cases); b) incorrect cytogenetic diagnosis (two cases); c) cytogenetic result description absent (two reports); d) incorrect interpretation (two reports).
We will show the EQA state of the art and the 10th round results.

PS19.27

Exploring health professionals' opinions of the All Wales Familial Hypercholesterolaemia Cascade Testing Service as a model for genetic service delivery

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Familial Hypercholesterolaemia (FH) is a relatively common disorder, with a high risk of mortality from coronary heart disease at a young age. FH can be diagnosed clinically and/or by identification of a pathogenic mutation. When FH is identified, early deaths can be prevented by administering serum cholesterol-lowering drugs.
The All Wales Cascade Testing Service, introduced in 2010, endeavours to systematically identify and treat affected individuals. Cascade testing is routine in genetic practice, but this service offers a novel way of delivering genetics services in collaboration with another medical specialty. Diagnostic genetic testing of individuals affected by FH is managed within the lipid clinics and cascade genetic testing of relatives is offered by the genetics service.
This study explored health professionals' opinions on the delivery of cascade genetic testing by a multidisciplinary service, and whether they consider this to be a good model for providing genetics input. Nine participants (three nurses, two genetic counsellors and four consultants) participated in semi-structured interviews. These were transcribed and thematically analysed.
Most (7/9) thought the FH Cascade Testing Service was an excellent model which worked well. However, many (5/9) felt that this level of service may not be necessary for a treatable condition and had reservations as to how well it would work if translated into other areas of the UK. It was suggested that it may be more appropriate for each specialty to include staff trained to manage inherited conditions, calling on the services of the regional genetics service if and when necessary.

PM19.28

How communication of genetic information within the family is addressed in genetic counselling: a systematic review of research evidence

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Supporting consultands to communicate risk information with their relatives is key to obtaining the full benefits of genetic healthcare. Guidelines

recommend that professionals should not directly contact family members and that they should actively encourage consultands to transmit relevant information to their relatives and support them throughout this communication process; however, there is lack of clarity on how this should be done. To understand how healthcare professionals address this issue in clinical practice and what interventions are used specifically to assist consultands in their communication of genetic information to appropriate relatives, we conducted a systematic review. Four electronic databases were searched for papers published, in English, January 1997 through May 2014. Hand-search in four subject-specific journals was also performed. Fourteen papers met the inclusion criteria defined.
Thematic data analysis has shown that dissemination of information within families is actively encouraged and supported by professionals. Three overarching themes emerged: (1) direct contact from genetic services: sending letters to relatives of mutation carriers; (2) professionals' encouragement of initially reluctant consultands to share relevant information with at-risk relatives; and (3) assisting consultands in communicating genetic information to their at-risk relatives, which included subthemes as (i) psychoeducational guidance and (ii) written information aids.
Findings suggest that professionals' practice and interventions are predicated on the need to proactively encourage family communication. We discuss this in the context of what guidance of consultands by professionals might be appropriate, as best practices to facilitate family communication, and of the limits to non-directiveness in genetic counselling.

PS19.29
How are genetic counsellors educated? A systematic review of the evidence.

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The need for appropriately trained genetic counsellors to support genetic healthcare is now acknowledged. However, while programs for education of genetic counsellors exist in a number of countries, these do not conform to any specific international standards. This systematic review was conducted to evaluate the publications for research of evidence regarding components of educational programmes for genetic counsellors worldwide. Databases were searched for studies published in English from 2000-2014 related to the topic. We identified 406 potential papers, of these 11 studies met the inclusion criteria. The findings indicate that, in general, the theoretical components of genetic counsellor programs conform to the recommendations and requirements of relevant professional bodies. However, clinical preparation of genetic counsellors in real-life professional practice settings seems to be less well addressed as this is essential to ensure genetic counsellors are able to provide safe patient care after graduation. Further work to gain agreement internationally on genetic counsellor education is needed.

PM19.30
An exploration of families' beliefs about the causes of psychosis or schizophrenia and whether they feel they will benefit from psychiatric genetic counselling.

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Introduction: Since the full spectrum of genetic variants that confer susceptibility for complex psychiatric conditions (CPCs) remains unknown, genetic testing and counselling presents challenges. The literature to date while incomplete has profound implications for our understanding of aetiology, family burden and disease-associated stigma, demonstrating the need for a psychiatric genetic counselling (PGC) intervention. Research into the experiences of affected families can help to surmount these barriers and improve patient experience, providing a foundation from which genetic counsellors can develop confidence in the value of their services, even in the face of uncertainty.
This paper explores family experiences and the potential for the development of a PGC service in the UK.
Methods: Using a qualitative approach; relatives of individuals with schizophrenia or a related diagnosis were asked a series of open-ended questions about their experiences, their beliefs about cause, and their views about genetic testing and PGC. The interviews were recorded, transcribed and thematic analysis was applied to the dataset.
Results: The majority of participants reported no interest in genetic testing but most of them thought that GC would be invaluable to understanding aetiology and the psychosocial aspects of CPCs. Overall, respondents believed that there is genetic vulnerability to developing CPCs and despite genetic testing being unavailable a PGC intervention may be of value to future generations.
Conclusion: Ultimately, these findings offer insight into the delivery of PGC, which could be applied to other areas of genetic counselling (GC) where uncertainty lies, with a downstream effect on future GC practice.

PS19.31
Talking about uncertainty - a communication skills training model for professional development.

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Rapid development in genomic technologies has increased the potential for uncertainty in the interpretation of test results. Talking with patients about uncertainty is more complex and challenging than ever. For some time, Communication Skills Training (CST) for professional development of genetic counsellors in NSW, Australia, has been successfully based on an exploratory learning model of facilitated role plays utilizing actors in comprehensive scenarios. However, evaluation of a 2012 CST workshop requested by genetic counsellors on 'talking about results of uncertain significance' found that 'uncertainty' was lost in role-play. Some participants reported a need for something tangible and to hear 'how others talk about it'. In 2014, the Centre for Genetics Education, NSW Health with the Pam McLean Centre, Sydney University, redeveloped the one day workshop to explore the concept of 'uncertainty' and work with participants' counselling experience to build a toolkit for practice. Two role plays, targeting consultation around 'results of uncertain significance' followed. Genetic counsellors (16) and geneticists (2) attended. Learning needs were met (89% entirely, 11% partially) including the opportunity to observe, reflect upon and apply different approaches to talking about uncertainty, relevance to practice (90%) and confidence. All were likely to refer back to the tool kit (100%). Two senior counsellors reported they had not thought about their practice this way before. A targeted approach to CST around 'uncertainty' that provides participants with tools as well as retains the benefits of exploratory learning may assist professional development of health professionals in this important area.

PM19.32
Genetic counsellors in Sweden - Their role and value in the clinical setting

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Genetic testing is becoming more commonplace in general and specialist health care and should always be accompanied by genetic counselling, according to Swedish law. Genetic counsellors are members of the multi-disciplinary team providing genetic counselling. We examined the role and value of genetic counsellors in Sweden, using a cross-sectional on-line survey. The findings showed that one main difference between a genetic counsellor and medical geneticist was that the doctor had the main medical responsibility. The genetic counsellors added value in the clinical setting by acting as the "spider-in-the-web", having a holistic, ethical and psychological view, being able to offer continuous support and build a relationship with the patient, and being more accessible than medical geneticists. Genetic counsellors in Sweden contribute substantially to the care of patients in the clinical genetic setting.

PS19.33
Genetics education in primary care - preference for an informal learning model

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Objective: The objective of this study was to explore Primary Care Providers' (PCPs) perceptions of genetics in primary care practice and their genetics education and resource needs.
Method: This was an exploratory, qualitative study using 10 semi-structured

interviews and 3 focus groups with urban and rural participants. Key informants for the interviews included a purposefully selected sample of family physicians, nurse practitioners, and health administrators. Volunteer family physicians participated in focus groups. Thematic analysis was used; data was interpreted using constructivist grounded theory. Use of a distanced interviewer, standardized protocol, and member checking assured consistency and trustworthiness of the data
Results: PCPs recognized the value of genetics, but issues around the extent to which genetics should or could be incorporated into daily practice were expressed. PCPs, particularly in rural areas, described being judicious in referring patients for consults and stated their role included an assessment of which patients could be managed locally. To support their decision making, a need for resources was identified. Preferred approaches across all PCPs included informal learning opportunities such as "virtual" hallway consults, web-based cases and Telehealth consults. Participants became interested in supporting further genetics education opportunities locally.
Conclusion: PCPs see value in genetics but identify challenges integrating genetics into practice. Identifying opportunities for informal learning has the potential to improve primary care genetics CME and to optimize PCPs involvement in genetic risk assessment, diagnosis and management. Success in incorporating genetics into daily medical practice has the potential to reduce the interval from presentation to intervention, resulting in improved health outcomes.
Supported by CTAQ endowment fund

PM19.34
Genetic Tests, Genetic Diagnosis and Bioethical Issues: Evaluation of Medical Student's Opinion

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The increasingly wide range of available genetic tests (GTs), understood as the analysis of hereditary characteristics using the techniques of molecular biology, biochemistry or cytogenetics, is used for the diagnosis of many genetic pathologies. These techniques, of growing sophistication and detail, allow a more accurate clarification of unclear medical conditions, but frequently produce "unexpected results" (UR) or "variants of unknown clinical significance". The diverse ethical problems that relate to GTs, their results, and deciding who may/should be informed of findings constitute a serious challenge to health professionals.
Integrated MSc Medical Students (74) from the Faculty of Medicine, University of Porto, Portugal, volunteered to answer the 13 questions of a questionnaire about the above-mentioned problems. The statistical analysis of results was done using SPSS, version 20. The social characterization of samples revealed that mean age of students was 21,44, mostly females (70,3%). Main results showed: 1- genetic counselling before/after GT was considered very important (69%/73%.); main concern as "professional" dealing with UR is user's anxiety (82,4%); UR should importantly be transmitted in case of mendelian disease (36,5%) and affected carriers (35,1%); it was very important (18,9%)/important (45,9%) to further investigate UR; only sometimes (63,5%) a more precise genetic diagnosis may be beneficial, although it would help to understand the disease (74,3%); the user, if competent adult, should decide if/when do genetic tests (68,9%).
With this preliminary investigation, the authors highlight the importance of bioethics studies as useful tools for medical training.

PS19.35
Kyoto Model of developing a human genetics education program in Japan

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[Introduction] Genetic tests are becoming common in Japan. The curriculum guidelines of primary, junior high, and high schools in Japan includes little information regarding human genetics. In order to improve genomic literacy in Japan, we are developing an educational program of human genetics.
[Methods and Results] (1)International comparison of human genetics education at school: We analyze the national curriculums of science in Japan and other countries, with particular attention to the genetics and human genetics in order to establish human genetics education program. (2) Exhibition of human genetics as a program of an entertainment for kids: More than 3000 kids and their families participated in the two-day event, and more than 500 come to our exhibition. Families studied DNA, gene and chromosome. (3) Lectures in a high school biology class: We conducted three 50-minute lectures on human genetics. We discussed several genetic subjects, including genetic tests and prenatal diagnosis. (4) Seminars on human genetics for primary schoolchildren: We hold eight seminars in a year for about 100 schoolchildren in total each time. Each four-hour seminar is composed of lectures, experiments and discussions.
[Conclusions] Our project is unique in that students in the postgraduate course of genetic counseling and certified genetic counsellors are leading this project. The main purpose of our project is to familiarize children and parents with human genetics. We have just started the program, and it will take time to evaluate our project. We are developing teaching materials. This work is supported by the Educational research fund of Kyoto University and Japan society for the promotion of science

PM19.36
Creating educational iPhone, Android and Windows smartphone multi-platform apps to facilitate understanding of clinical genomics and related terminology

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Introduction:
A high proportion of postgraduate students now use Apple (iOS), Android or Windows smartphones. Mobile internet access is, however, often intermittent or slow (eg on public transport). Moreover, online information is often unhelpful, particularly in relation to complex topics (such as clinical genomics terminology). Smartphone apps (applications) can provide user-friendly alternative information sources.
An app was created to provide easily accessible, comprehensible and illustrated explanations of commonly used (but often confusing) clinical genomics acronyms, terms and processes (eg "FASTQ", "BVCF", "GVCF", "IGV" and "Bowtie2"), for students and professionals. An interactive quiz app based on the same material was also created, permitting self-assessment.
Materials and Methods:
A cross-platform application programming language was used, with Apple iOS and Android software development kits (SDKs), to build device-specific apps. Creating iOS apps also required an Apple iOS Developer Program Licence and the latest Apple Xcode software.
Results:
An illustrated, indexed, searchable and advertisement-free, educational app plus an accompanying self-assessment quiz app were created and tested on Android, iOS and Windows. Using the same programs and licences, the authors recently also created several medical genetics self-assessment apps (that have already been used by 273 undergraduate medical students with 978 total downloads). Anonymous feedback collected from the students has been 100% positive.
Conclusions:
It is hoped that these clinical genomics apps will be found useful by genetics students (and perhaps even by professionals). Although their creation is complex, smartphone apps represent a convenient and quick means of obtaining information and of enhancing learning.

PS19.37
Workforce Transformation: Health Education England's Genomics Education Programme

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The UK aims to become the first country to introduce whole genome sequencing into its mainstream healthcare system to aid clinical decision making, and has launched the 100,000 Genomes Project (www.genomicsengland.co.uk) to pave the way in this area.
Health Education England (HEE) has responsibility for improving the quality of care of patients, through education, training and the personal development of all staff within the National Health Service (NHS) in England. We have therefore established an ambitious Genomics Education Programme (GEP) to support the 100,000 Genomes Project and prepare the NHS for a future in which genomic medicine is fundamental to the patient pathway (www.genomicseducation.hee.nhs.uk)
The programme is comprehensive in addressing the training and educational needs of the highly specialised, specialised and general workforces. As part of this, we are:
• devising e-learning and workshops to support each step of the 100,000 Genomes Project pipeline;
• providing a Master's in Genomic Medicine for up to 550 medical, scientific

and nursing staff, plus online CPPD modules for all staff;
• commissioning 28 higher specialist training (HSST) posts in Genetics and Molecular Pathology, with six more planned for Clinical Bioinformatics; and
• developing awareness-raising courses and videos suitable for all NHS staff.
Early evidence that our approach is effective is reflected in 600+ registrations for our first two online courses, with 200+ completions. The programme is also very active on social media, with 2,500 Twitter followers and a monthly retweet reach of 85,000.

PM19.38
RARE-BestPractices - supporting best quality care for rare diseases

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Over 30 million Europeans are rare disease (RD) patients. RD are defined as affecting no more than 5 per 10000 persons and there are approximately 6000 recognised rare conditions, many with a genetic basis. Diseases are often life threatening or chronically debilitating and present a significant burden to patients, carers and service providers. Access to best care across the European Union (EU) is inconsistent and limited by proximity to disease experts, insufficient evidence for decision making and clinician's lack of experience of low prevalence conditions.
RARE-Bestpractices is a four year project delivering an online platform to improve care management through the sharing, appraisal and development of RD best practice, knowledge and information. The project focus is to collect, evaluate and disseminate existing RD guidance, to cultivate a robust methodology and suite of tools appropriate to RD guideline development, and to facilitate collaboration, knowledge exchange and research in the area of RD.
Collection development is underway to populate new RD guidelines and research recommendation databases. The guideline database will facilitate appraisal of new and existing guidance using AGREE II methodology to support easy identification of best quality clinical guidance for clinicians, patients and their carers.
RDs affect a large number of the population. By bringing together stakeholders from across the EU the project will deliver a suite of RD management tools from a single access point to improve equity of care in this patient group.
The RARE-Bestpractices project is funded by the European Union's Seventh Framework Programme. Project Ref.: n° 305690.

PM19.40
Streamlining services to ensure equality of care for patients with or at risk of Haemoglobinopathies in Cardiff

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The All Wales Medical Genetic Service and Sickle Cell and Thalassaemia Service have been working together since 2013 to ensure equality of care for patients found to be carriers of or affected by Haemoglobinopathies. This has required multidisciplinary working between Genetics and Haematology and a comprehensive service is now being delivered to these patients. Pathways were devised for four areas; antenatal, non-urgent, possible Alpha Thalassaemia carriers and affected individuals.
Antenatal: Patients with abnormal/unusual haemoglobin screening results were referred to Medical Genetics via the antenatal clinic. Partner testing was arranged where requested and high risk couples offered prenatal diagnosis or neonatal testing.
Non-urgent: All carriers of clinically significant Haemoglobinopathies were seen routinely by Specialist Genetic Counsellors in the genetics clinic. Those who required further testing to confirm the carrier state were not seen until molecular testing had been carried out. This allowed the results to be explained in full without the need for further appointments.
Possible Alpha-Thalassaemia Carriers:
Due to the high carrier frequency of possible Alpha Thalassaemia, with up to around 17% of the world population being carriers, it is neither necessary nor possible to confirm all carriers. A standard letter was drawn up inviting those from areas with a high prevalence of Alpha Zero Thalassaemia, namely South East Asian or Mediterranean descent, to contact us. A patient information sheet was provided for all others.
Affected: It was agreed that affected individuals would be seen directly by the Consultant Haematologists and the Sickle Cell and Thalassaemia Nurse Specialist.

PS19.39
100 recorded interviews with human geneticists

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Between 2003 and 2014 the author has undertaken 100 recorded interviews with older human and medical geneticists from a wide range of countries, mainly European. Most of the interview transcripts are now available on the www.genmedhist.org website (also now accessible via the ESHG website), and they provide a wide-ranging insight into the origins and early development of the field that complements its written history.
Prominent themes include the early applications of human cytogenetics, the beginnings of human molecular genetics and the development of clinical and laboratory genetic services in different countries.
The interviews give a vivid picture of many of the founders of human and medical genetics, including much unpublished information on their early work and lives. Descriptions by those interviewed of their own teachers and mentors extends this information back in time to those already active before World War 2.
The challenge of extending this project and ensuring that it continues to cover younger generations of workers is now being taken up by ESHG and will hopefully provide a valuable resource for future historians and for all who wish to learn how human and medical genetics have developed worldwide, and especially across Europe, from their beginnings to the present time.

PS19.41
Family Communication in Inherited Cardiovascular Conditions in Ireland

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ABSTRACT: Over 100,000 individuals living in Ireland carry a mutated gene for an inherited cardiac condition (ICC), most of which demonstrate an autosomal dominant pattern of inheritance. First-degree relatives of individuals with these mutations are at a 50% risk of being a carrier, disclosing genetic information to family members can be complex. This study explored how families living in Ireland communicate genetic information about ICCs and looked at the challenges of communicating information, factors that may affect communication and what influence this had on family relationships.
Face to face interviews were conducted with nine participants using an approved topic guide and results analysed using Thematic Analyses. The participants disclosed that responsibility to future generations, gender, lack of contact and proximity, all played a role in family communication. The media was cited as sources of information and knowledge of genetic information tended to have a positive effect on families. Results from this study indicate that individuals are willing to inform family members, particularly when there are children and grandchildren at risk, and different strategies are utilised. Furthermore, people do not live in vacuums and their understanding of genetics is partially regulated, not only by their families but by the way society handles information. Therefore, genetic health professionals should take into account the familial influence on individuals and their decision to attend genetic services, and also that of the media.

PM19.42
Premarital genetic counseling for a familial mutation as a family initiative towards prevention of Lamellar Ichthyosis: a five year experience

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Targeted testing for a known familial mutation can be a powerful tool in countries with limited options for recurrence risk reduction or prevention such as termination of affected fetus. Sultanate of Oman is one of the Islamic countries that follow regulations against termination of fetus unless for maternal indications. Preimplantation genetic diagnosis is allowed under regulations but not available within the country. Considering the fact that family intermarriage is favored, providing premarital genetic counseling and testing for individuals at risk of familial disease should aid risk reduction. A highly inbred family with autosomal recessive Lamellar Ichthyosis has had a known familial mutation in the TGM1 gene since 2008. Despite the availability of the test the number of individuals considering carrier status testing was unremarkable for four years. The main barrier of premarital testing is the geographic distance to the testing center, where the family have to travel thousands of miles for counseling and testing. Avoidance of social and cultural stigma had also discouraged the testing. In the last two years, three new births of affected children from distantly related parents facilitated the

family affinity towards testing; hence number of individuals attended the clinic for carrier status counseling and testing has dramatically increased. To date, 22 cases had attended for genetic counseling and testing in comparison to seven before the new births. There are still barriers to overcome so the family can access the service effectively; like distance travelling and cost. Training a genetic nurse or organizing mobile genetic counseling clinic might facilitate better premarital planning and avoidance of new cases.

PS19.43
Management of MCA/MR through a local service network in Languedoc-Roussillon region (France).

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Multiple congenital anomalies/mental retardation (MCA/MR) involve the physical: malformations, growth disorders, sensory difficulties,⋯ and « cerebral »: neurological, cognitive difficulties, behavioral troubles development of an individual. They affect close to 3% of the population and account for over 3000 rare disorders. Management of patients is multidisciplinary and goes beyond the simply medical domain. In addition to doctors many other healthcare professionals are involved at an early stage : physiotherapists, medico-social and education personnels.
In 2009, to meet the needs of patients and their families with such rare diseases leading to MCA/MR, the VADLR (Live with a Developmental Anomaly in the Languedoc-Roussillon) health network was created to weave a web of practitioners and institutions tightly around patients. The mission of the network is to
-contribute to the development of an efficient and coordinated local structure to accompany patients with a developmental anomaly,
-increase the knowledge and training of professionals and patients,
-facilitate access to information,
-direct towards proper care and facilitate access to legal rights,
-contribute to coordinating the actions of all players involved in complex situations.
After 5 years, assessment is positive: for 2014 we recorded 3130 calls,15 963 internet connections (http://www.anomalies-developpement-lr.net), 138 families accompanied. Since the creation of the network, more than 1200 professionals have been trained.
The increasing solicitation of this regional local service network signifies its usefulness to patients and professionals.

PM19.44
„Mendel's city" Brno celebrate Mendel's legacy - 150 years of the genius of genetics

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In 2015 we are commemorating 150 years since Mendel's lectures in Brno. Gregor Johann Mendel presented on the 8th of February and 8th of March 1865, for the first time, the results of his research at a meeting of the Natural Science Society in Brno. His perceptive observational talent and use of mathematics stood behind the defining of three principles, often referred to as Mendel's principles of heredity.
Mendel Museum with the great support of many Czech and foreign partners celebrate this important anniversary with exhibitions and conferences.
In cooperation with The Czech Society of Medical Genetics, Department od Medical Genetics, University Hospital Brno, Medical Faculty and Faculty of Science Masaryk University Brno we organize „The week of human Genetics" with lectures for the public „Unveiling the secrets of the human geonome", The Student Scientific Conference on Biomedicine , The National Congress of the Society of Medical Genetics, Czech Republic, The 48th Annual Cytogenetic conference and the conference „Human Genetics from Mendel to the Present Day". Other conferences: Research in plant genetics (From Mendel's peas to the present), The Darwin Day 2015 and Mendel Lectures 2015 and exhibitions: Hugo Iltis - the first Mendel´s biographer, The Construction Set Of Life. For the 150th anniversary of Mendel's laws in State Darwin Museum in Moskow and „Unseen for many years" a unique exhibition of Mendel´s original documents prepared in cooperation with University of Illinois.
The Anniversary is held under the auspices of Prime Minister of Czech Republic, Rector of Masaryk University Brno and The Czech Committee of UNESCO.
www.mendelgenius.com

PS19.45
Neonatal screening: a historical-comparative perspective

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After the first regional initiatives to screen newborns for PKU in the United Kingdom and United States in the 1960s, several European countries set up screening programmes. Though the number of disorders screened for gradually increased, European countries differed in their choices for specific screening strategies and the number of disorders screened for: from 1 to 29 (Loeber et al., 2012). For instance, in case of Congenital Hypthyroidism, the Netherlands followed Canada and several US states in the strategy used to detect both primary and central forms of CH, while most European countries concentrated on the primary forms. The Netherlands was one of the first countries to introduce screening for Congenital Adrenal Hyperplasia (in 2000). The reasons for this variety may range from health care priorities and budgets to practical considerations, organisational constraints or, perhaps, chance. Pollitt (2006) has suggested that the differences may be related to the professional background of individuals involved in policy-making. For the US the influence of patient organisations and commercial parties has been mentioned (Paul & Brosco, 2013) . For the Netherlands we did not find evidence that these latter forces have played a major role.
In our (poster) presentation we will highlight our research on the expansion of the Dutch programme after its beginning in 1974 (Loeber & Van El 2014). In addition we present an initiative to stimulate historical-comparative research on the rationale for the different choices that have been made regarding the expansion of neonatal screening in Europe.

PM19.46
Genetic counseling difficulties due to novel mutations in patients with Neurofibromatosis Type I.

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Neurofibromatosis Type I is one of the most common autosomal dominant disorders. It is caused by mutations of the NF1 gene. Disease penetrance is about 100%, while patients present mutational, allelic or phenotypic heterogeneity. Clinical features include café-au-lait spots, skinfold freckling, cutaneous or plexiform neurofibromas, optic gliomas and Lisch nodules of the iris.
Molecular diagnosis includes multi-step PCR, sequencing of all exons of NF1 and multiplex ligation-dependent probe amplification. The protocol was validated in a cohort of 80 NF1 patients and their relatives, identifying the germline mutations in the most of the cases. The effects of these mutations were assessed in combination with the clinical phenotype and using in silico analysis.
Our results include 28 known and 23 novel variants in coding and non-coding regions. The majority of novel mutations included stop codon mutations and small insertions or deletions. We also found whole NF1 gene deletion at two unrelated patients with severe clinical manifestations. Novel variants were analyzed with bioinformatic tools; "PolyPhen2", "SIFT", "Pmut", "Mutation Taster", "Mutation Assessor", "Provean v.1.1" and "Human Splicing Finder", and family segregation analysis was assessed from available family members. Evaluation of novel and de novo missense mutations is more complicated. The factors taken into account are the type of amino acid change, the sequence conservation across species and the in silico output of at least three different prediction tools. More specific, our work underlines the high frequency of novel mutations and additionally confirms phenotypic heterogeneity even within the same family, which complicates genetic counselling especially in cases of an ongoing pregnancy.

PS19.47
Role of genetic nurses in the Sultanate of Oman: Achievements and challenges

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In Oman, the Genetic and Developmental Medicine Clinic (GDM) was officially opened on December 6th 2011.The GDM is one of the specialty clinics in Sultan Qaboos University Hospital, which started with one geneticist physician and three staff nurses including one clinical nurse specialist. Clinical Genetics is a highly dynamic field with constant evolution and there was an

urgent need to involve the Omani nurses to contribute to the Omani healthcare. To meet with the growing demand and to provide quality care to the patients, the clinic expanded to involve four specialized physicians in clinical genetics, four clinical nurse specialist and genetic counselors. The genetic nurses adopted several tasks including the following: obtaining detailed family history and constructing a pedigree. Providing genetic information and psychosocial support to individuals and families for patient considering genetic testing, couples who have had multiple miscarriages and pre-marital genetic testing. The nurses were also involved in counseling families with known metabolic disorders, developmental disorders and hereditary cancer syndromes. Being a genetic nurse in Oman was associated with some challenges: the high rate of consanguinity and high numbers of patients with autosomal recessive disorders, low public awareness about genetic disorders, dealing with old cultural believes and denial and limited training in genetics at the nursing schools. Despite having all those challenges and the complexity of the tasks taken up by the genetic nurses, we have managed to help many families by learning from other staff members, attending courses and getting involved with the weekly multidisciplinary meetings.

PM19.48

An online tool to train non-geneticists how to consent cancer patients for BRCA testing

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Genetic testing for BRCA1 and BRCA2 (termed 'BRCA') has increasing implications for the optimal management of cancer patients, impacting on treatment and ongoing cancer surveillance and providing useful information for the wider family. NGS has made BRCA testing faster and cheaper. However, the gene testing pathways in most countries have not adapted to serve the potential increased throughput and remain focussed on the needs of unaffected at-risk individuals rather than cancer patients. To address these issues we have developed an oncogenetic BRCA testing pathway whereby members of the cancer team consent eligible cancer patients for BRCA testing after completing 30 minutes online training.

The training resources are fast, flexible, robust and highly accessible. They include e-learning videos (on YouTube) and comprehensive supporting information, all delivered via a simple online system. The resources empower the cancer team to take ownership of their learning and provide support and guidance from the genetics team where required.

To date 57 clinicians in the Royal Marsden cancer units have completed training during a wider, successful implementation of the oncogenetic gene testing pathway. 93% rated the training as "Good" or "Excellent". Feedback from patients consented by cancer team members that had completed the training showed that >95% felt well informed.

The training resources are now freely available at www.mcgprogramme.com/brcatesting.

Furthermore, the principles can be readily adapted for other genes / diseases for which a mainstream model of gene testing is applicable. This work was undertaken by the MCG programme, funded by the Wellcome Trust Grant 098518/Z/12/Z.

PS19.49

The Benefit of hindsight - the views of early adopters regarding preparation for personal genome testing

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Due to increasing availability of personal genome sequencing (PGS) there is a need to explore preparation of individuals pursuing testing. Potential participants were those who undertook PGS offered by Illumina Inc. through the first Australian Understand Your Genome Event in April 2014 and were recruited through their referring clinical geneticist. Seven, all professionals with genetics expertise, have been interviewed to date (RR 48%). Results included an autosomal dominant condition (NF1) not previously clinically identified; carrier status for recessive condition(s); a number of variants identified as likely pathogenic and many of uncertain significance; and pharmacogenetically relevant mutations. Themes identified included: 1). Rationale for being an early adopter. Most were motivated by objective professional interest and curiosity, without anticipating personal or family impact. 2.) Barriers. These included skepticism of colleagues, family members and privacy concerns. 3.) Pre-testing information and consent. Positive counselling experiences were reported with clinical geneticists. 4.) Impact of test result over time. Despite an initial objective motivation for testing, all expressed surprise at subjective impacts of the results. Several who initially perceived their results as "boring", later recognized their relevance, as health problems developed or family history was interrogated more closely. One participant with a known deletion causing carrier status, not identified by PGS, was concerned that a lay person may misinterpret the result. Disclosure of results has been limited. 5.) Reflection. All had no regrets about having PGS. However, in hindsight, participants felt certain issues needed greater emphasis at the pre-test session: expectations; residual risk; changes in interpretation with developing phenotypes; and personal and family impact and communication.

PM19.50

Encounters with prenatal and pre-implantation genetic diagnosis: Are we really offering a holistic clinical genetic service in Oman?

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The incidence of congenital anomalies and/or genetic disorders in the Omani population has reached figures greater than double the global statistics. Prenatal diagnosis (PND) and pre-implantation genetic diagnosis (PGD), contributing elements to a holistic clinical genetic service, provide at-risk parents with options to prevent the birth of an affected child. PND can be utilized by Omani couples, as long as the termination of pregnancy (TOP) is for a severely affected fetus, and occurs prior to the first 120 days. However, the practice of this procedure is impeded by the lack of services able to offer TOP, while PGD services are only accessible by travelling to international centers.

The increase in the number of requests for PND and PGD calls for an open ethical and legal discussion around this topic. We therefore elected to gather descriptive data from patients requesting PND/PGD over a two year period (2013-2014) to portray and examine the current situation faced by Omani couples. Twenty-three cases were identified and included in the study. The service user's perspective on PND and PGD, test results and patient driven outcomes together with the identified benefits and limitations of each are compared and contrasted. Interviews additionally provide data on the impact of the decision for the couple and their family and the effect of undertaking the procedure, on future pregnancy-related decisions.

A multitude of logistical, religious and psychosocial challenges are faced by patients. Awareness of the current situation will be extremely valuable in improving the service according to the patient's needs

PS19.51

Improving access to psychological services: Evaluation of a pilot project in Manchester Centre for Genomic Medicine

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The Improving Access to Psychological Therapies (IAPT) initiative in England aims to support the implementation of NICE guidelines for people suffering from depression and anxiety disorders. Psychological Wellbeing Practitioners (PWPs) have been located in primary care and NHS clinics such as Diabetes and Respiratory Medicine to provide low intensity psychological interventions based on a collaborative care model. We report an evaluation of a pilot project, supported by NW Health Education England, to look at the acceptability of an IAPT practitioner offering a weekly clinic within a Regional Genetic Centre.

24/40 patients referred to the IAPT practitioner by genetic counsellors between January and May 2014, took up the offer of psychological support. Of these 24 individuals; 8 received between 2-6 counselling sessions and reached recovery, 9 were signposted to local psychological services, 7 opted for telephone contact and 2 dropped out. The PWP contributed to teaching sessions within the department and helped with triaging and signposting of those patients requiring additional psychological support. Qualitative interviews with 8 genetic counsellors revealed that the most effective element of the project was the part it played in facilitating referral pathways that improved the access to local psychological services. The genetic counsellors were all very positive about the benefits of having access to specialist mental health advice from the PWP, who was able to see a small caseload of patients and advise on availability of community and online counselling services to the genomic medicine team.

PM19.52

A multiple case study approach to patients and caregivers as sources of innovative ideas and solutions

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Introduction: Recent academic literature shows that patients and caregivers are a significant source of innovative solutions related to their medical condition. To date, little is known about the process by which these innovations emerge, how they diffuse, and how they impact the lives of patients and caregivers.
Objectives: We followed a multiple-case study approach to map a set of patient innovations and adoptions of patient innovation cases, and systematically explore how and how far patients and caregivers innovate in the health care field. In addition, we propose some explanations for why patients and caregivers stop at a certain stage of progression of the innovation process.
Material and Methods: We conducted 15 extensive semi-structured interviews with patients and caregivers of the following group of diseases: spinal cord injuries, Angelman syndrome, epidermolysis bullosa, cerebral palsy, and hemiparesis. These individuals shared their disease experiences and their efforts, or the lack of them, to overcome specific health problems. This includes 4 "holistic" case studies and 26 "embedded" case studies. We analyzed patient innovation paths and present them in the fall-offs conceptual framework.
Results: Through a cross-case analysis, we find that duration of the disease, complexity and pressure of a certain situation, belonging to a group or a community, and perceived value of a solution are among the most important reasons that impact how far patients and caregivers take their innovations.
Conclusion: As a result of our multiple-case analysis we present a set of propositions from which future research in the field is warranted.

PS19.53

Rare diseases - Romania progresses in the last years

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Introduction. Rare diseases began to be a priority for patients and subsequently for patient support groups in our country after 2005. Doctors were called to join them and mixed organizations have delayed occur. ANBRaRo Alliance was the first NGO to bring real changes in terms of attitude and then bringing a change in Ministry of Health approach.
Method. We used data provided by the 6 existing clinical genetics centers placed in university cities from our country, with doctors working in this field and with NGOs established in support of different groups of patients with rare diseases.
Results. The last decade has been one that has completely changed the dates of this issue in Romania. These diseases have actual opportunity to be treated in small numbers but the way was opened and National Health Insurance House are dedicated annually to these patients. The actions were guided by the rules of the EURORDIS and interdisciplinary was a vital principle. The only major deficient is the diagnosis system.
Several actions sensitizing the public about the area were used - The Romanian Rare Disease Day campaign, conferences for patients, courses for Journalists, media materials.
Conclusions. The road will include other collective efforts and current patients are still deprived of all complex diagnostic and therapeutic approach required by this area, but coming years must bring the same rate change for this class of diseases. The role of patient organizations as the engine of initiatives in this area was decisive.

PM19.54

Patient innovation under rare diseases and chronic needs

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Introduction: Patients afflicted by rare diseases (RR) often need to develop innovative solutions to cope with the disease. The impact of this innovative capacity and the driving factors for innovation in patients with RR are not clearly understood. The objectives of this work were to measure frequency of RR patient innovation; to measure efforts by patients to share their solutions; to explore which factors drive patients to come-up with solutions and share them with others.
Material and Methods: A questionnaire grounded in user innovation theory was adjusted to RR context and 500 patients were surveyed. Two medical professionals validated solutions reported by patients and multivariate regression models were developed to test relationships between our key variables and patient innovation and solution sharing.
Results: 263 of respondents reported having a solution; 46 reported solutions that they personally find valuable, and that are also evaluated as novel by expert medical evaluators. The likelihood that patients innovate increased with education level and with increase in perception of limitations imposed by the disease. 84 individuals shared their solutions, and the most common mode of sharing was patient-to-patient (74 individuals). There was a positive relationship between the impact of a solution on the respondents' overall quality of life and likelihood of patients sharing their solutions, and an inverted relationship between age and the solution sharing.
Conclusion: The innovation and solution sharing characteristics observed in our study may be a tremendous potential resource of information to improve management and care for many who are similarly afflicted.

PS19.55

Referral pathways to the Department of clinical genetics within a Czech Republic teaching hospital.

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Timely referral to a clinical geneticist depends on the knowledge of referring clinicians of the scope of clinical genetics services. We set out to map who are our referring clinicians based on the structure of our consultations.
The Department of Clinical Genetics of the Teaching Hospital Olomouc serves a population of about 920,000 inhabitants and is staffed by 3.1 clinical geneticists. The hospital is one of eleven teaching hospitals in the Czech Republic and the sixth largest. It has 1200 beds and 50 departments.
In total 1584 genetic consultations were performed by the Department in 2014 (510 consultations per clinical geneticist). We divided them according to the prevailing diagnosis into paediatric (305, 19%), related to infertility (225, 16%), related to ongoing pregnancy (417, 26%), oncologic (327, 21%), neurologic (327, 21%) and others (191, 12%).
A large proportion of consultations were requested by the doctors from within the hospital. This may reflect the specialist care the hospital provides and the structure of the health care in the Czech Republic, where primary care physicians usually refer the patients with more complicated problems to a specialist, who then deals with their further care. It may also reflect on the limited knowledge of the possibilities of genetic testing by primary care physicians including the referral pathways, indications for and the benefits of genetic testing.
This finding has implications on targeting continuous postgraduate medical education of clinical genetics in the Czech Republic.

PM19.56

Evaluation of reimbursement systems of genetic tests in the NGS era and health care costs planning of NHS: urgent need in 2015

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Next Generation Sequencing has improved genetic testing but also analysis of molecular biomarkers in medicine and in particular in oncology.
To update the reimbursement system for laboratory tests is an urgent need both in US and Europe. US Medicare health insurance announced that "laboratories performing test panels (i.e., tests for multiple biomarkers ordered together and completed on a single sample) must begin to register each panel under the MolDx Program". This means that "laboratories must obtain a unique MolDx identifier for each panel and bill such panels with a single CPT code. Currently, many laboratories submit these multiple biomarker "panels" as individual tests, providing a CPT code for each biomarker included in the panel".
In Italy the NHS is updating the reimbursement system and it is going to charge panels of tests that will be useful to answer a diagnostic question and not any more the single gene test.
We have performed a systematic analysis of activities and costs needed to perform genetics tests and we have identified a number of indicators to assess the workload. All these parameters have been incorporated into software to compare the performance activity and to evaluate performance within the same laboratory or to perform a benchmarking with other laboratory in the country. Activity-Based Costing is the methodology used to assigns the cost of each activity. The evaluation of costs and performance could be a way to establish a correct public health policy and to evaluate clinical utility and costs/benefits.

PS19.57

Different ways of multi-disciplinary working. The results of a prospective observational cohort study of referrals to a UK regional genetics service.

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Differing genetic service delivery models exist across Europe with clinicians developing innovative ways of working to meet demand.

This paper presents the first empirical prospective observational cohort study of UK multi-disciplinary genetic service delivery. It describes and explores collaborative working practices including the utilisation and role of clinical geneticists and non-medical genetic counsellors. Six hundred and fifty new patients referred to a regional genetics service were tracked through 850 clinical contacts until discharge. Referral decisions regarding allocation of lead health professional assigned to the case were monitored, including the use of initial clinical contact guidelines. Significant differences were found in the cases led by genetic counsellors and those led by clinical geneticists. Around a sixth, 16.8% (109/650) of referrals were dealt with by a letter back to the referrer or re-directed to another service provider and 14.8% (80/541) of the remaining patients chose not to schedule an appointment. Of the remaining 461 patients, genetic counsellors were allocated as lead health professional for 46.2% (213/461). A further 61 patients did not attend. Of those who did, 86.3% (345/400) were discharged after one or two appointments. Genetic counsellors contributed to 95% (784/825) of total patient contacts. They provided 93.7% (395/432) of initial contacts and 26.8% (106/395) of patients were discharged at that point. The information from this study informed a planned service re-design. More research is needed to assess the effectiveness and efficiency of different models of collaborative multi-disciplinary working within genetics services.

Grant support: Liverpool Women's NHS Foundation Hospital Trust Charitable Grant.

PM19.58

Communicating genomic research results to patients: our experience from the Developmental Genome Anatomy Project (DGAP)

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DGAP is a collaborative research study that serves to annotate the human genome. Participants are recruited with apparently balanced chromosome rearrangements and abnormal phenotypes and Whole Genome Sequencing (WGS) using our jumping library approach is employed to determine chromosomal breakpoint regions. We validate pathogenicity of disrupted and/or dysregulated genes using cellular or animal models.

Interpreting diagnostic significance of identified genomic regions sometimes generates information of clinical relevance for participants. However, little consensus exists as to what research studies do with their findings, particularly if results are of uncertain clinical significance, and an historical approach has been not to return any information. Here we use DGAP cases to discuss the potential benefits or harms to participants if such research results are communicated.

First, we describe a case where we delayed reporting research results for several years whilst we sought validation of our findings. The family independently pursued their own investigations through online searches, eventually leading to suspicion of the same candidate gene. We discuss the impact of our delay in sharing our research results with the family and the value in directing the validation testing.

We also present two prenatal cases where the fetus was found to have a *de novo* balanced rearrangement early in pregnancy. We used our WGS approach to report results to the parents within two weeks of referral. We discuss the impact our research results had on their decisions concerning their pregnancies, describing the ethical dilemmas that arose from both research and clinical perspectives.

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PS19.59

YouTube, animation and genetic education

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We have developed a number of short videos, some animated, to help develop integrated online genetic education. Our vision is to develop core videos on topics such as pedigree drawing, genetic cascade and predictive testing, exome/genome sequencing, incidental findings and ethics, relevant to practises in Ireland. Outside of the core, we have specialist topic videos relevant to genetics, created by colleagues from diverse disciplines. Our target audience are health care professionals from all aspects of mainstream medicine.

Much of our content, such as our six animated videos, are freely available on YouTube. These videos already have ~20,000 views. Families can view them prior to an appointment.

We found that short videos (~5 minutes) are more popular; the viewer stays with the video through its entirety in contrast to long videos. As viewers post thumbs up and thumbs down as well as comments, this is a useful way of gaining prompt feedback. Our two recent chromosome translocation videos have already had 6,000 views and are being linked in with Unique, the rare chromosome support group. We are translating these videos into ten languages to increase applicability. http://bit.ly/RecipTranslocation & http://bit.ly/RobsTranslocation

Feedback includes:"Wow this video was more helpful than any other genetic video on Youtube" & "Now I understand it thanks".

Whilst YouTube is used by the public to access genetic information, much of the educational content is aimed as researchers. There is a market for simple genetic information to be developed for the public.

Grants; UCD; Temple Street children's fund for Health; Shire Pharmaceuticals

PS20.01

Ethical, legal and societal implications of biobanking at European level: a Common Service of the European biobank and biomolecular research infrastructure

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BBMRI-ERIC - Biobanking and BioMolecular resources Research Infrastructure - European Research Infrastructure Consortium is a unique infrastructure where >16 member states have joined forces to setup a Pan-European distributed research infrastructure in order to facilitate the access to biological resources and facilities and to support high quality biomolecular and biomedical research. Common Services (CS) form a key element of the infrastructure. The proper consideration of ethical, legal and social issues (ELSI) is key to any biobanking activity. The CS ELSI aims to facilitate and support cross- border exchanges of human biological resources and data attached for research uses, collaborations and sharing of knowledge, experiences and best practices. This is particularly relevant for human genetic research where biobanking is a vital element.

Methods and Materials

Following a preparatory meeting and a call for tender in 2014, a Common Service ELSI was established starting in February 2015.

Results

The missions of this CS are, in the domain covered by BBMRI-ERIC:

Ethics check of research proposals submitted to BBMRI-ERIC ;

Monitoring of ELSI issues;

Policy: follow up relevant evolution in legislations/regulations and public consultations at European level;

Advising and Help-desk: provide updated background information and guidance regarding ELSI, towards harmonisation;

Dissemination: of results of relevant surveys and studies ;

Tools development: organize tools and services to address ELSI ;

Experience sharing regarding ELSI, notably data protection ;

Education: specific training.

Conclusion

The CS ELSI is addressing many of the ELSI preoccupations of geneticists and collaboration with ESHG may be of interest.

PM20.02

Identification of men with a genetic predisposition to prostate cancer: targeted screening of BRCA1/2 mutation carriers and controls. The IMPACT study Quality of Life Study

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The IMPACT study is recruiting male BRCA1/2 mutation carriers and controls (tested negative for a mutation), to undergo annual PSA screening +/- prostate biopsy. In addition to the screening protocol 15 centres are running a quality of life (QoL) study which aims to: (1) evaluate the psychosocial impact of screening for prostate cancer (PrCa) in terms of (a)cancer worry, (b)distress of screening and (c)health related QoL; (2) identify factors associated with negative psychosocial outcomes; (3) determine compliance with screening over time.
Methods: Men completed a questionnaire prior to each annual screening visit. The questionnaires included sociodemographic data and the following measures: HADS, IES, SF36, MAX-PC, Cancer Worry Scale, self reported risk perception and a knowledge questionnaire (understanding of BRCA1/2 and PrCa). The results of the baseline questionnaires are presented.
Results: In total 484 men enrolled in the QoL study and uptake was 82-100% at participating sites. Mean scores for HADS and SF36 were within reported population norms and mean IES scores were within normal range. No statistically significant differences were observed between groups. Average knowledge score was 77%, demonstrating a good understanding. Mean MAX-PC scores were low suggesting a low level of prostate-cancer specific worry. Cancer worry levels were low and BRCA2 carriers in particular perceived their risk of PrCa to be higher than average.
Conclusions: Uptake of the QoL study is high in participating centres. Baseline psychosocial measures suggest a low prevalence of distress and participants demonstrate a good level of knowledge about PrCa and the BRCA1/2 genes.
Grant support: Cancer Research UK, McAulay Foundation, NIHR Biomedical Research Centre

PS20.03
Managing genetic testing in childhood

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Traditionally geneticists have urged caution in genetic testing in childhood in order to preserve autonomy. Advances in medicine mean that a greater number of tests are being carried out that have a predictive component. With greater awareness of genetic status from screening programmes and genomic testing we can no longer advocate that genetic testing should only be undertaken in exceptional circumstances.
The lack of a robust evidence base for the long term psychosocial outcomes leaves the decision to test at the discretion of the geneticist. The concept of best interest is unclear and there may be a conflict of interest between parents and child. When should testing be carried out at the request of the parents, how and when should testing be discussed with a child and how and when should results be communicated subsequently.
We have reviewed all cases in our department where predictive or pre-symptomatic genetic testing has been carried out in childhood and examined the factors influencing the decision to test.
For predictive testing in all cases the parent requesting was the mother even when the affected parent was the father. Testing was more likely to be requested and undertaken where there were traumatic family circumstances and often in an attempt to end the impact of the condition on the family. Testing was often carried out ultimately because of fear of harm to the patient-professional relationship.
We present details of the cases learned and discuss the ethical framework and legal landscape.

PM20.04
Public attitude towards self-government of consumer targeted genetic testing in Japan

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Background: Japanese ministries are considering regulations governing DTC genetic testing. The METI has endeavored to set recommendations to quality control, scientific evidence, and informed consent, which allow self-governance. On the other hands, the MHLW thoroughly review the definitions of "diagnosis" to regulate DTC genetic testing. Our aim was to clarify public attitudes toward scientific evidence and self-government of DTC testing.
Methods: In March 2014, an anonymous online survey was administered to 24,718 men and women aged 20-69 years in Japan. The questionnaire included questions concerning genetic knowledge, attitudes toward genetic testing and the regulations governing DTC genetic testing.
Results: 7,540 individuals completed surveys. The mean age of the respondents was 45.7 ± 14.0 years. With respect to questions regarding willingness to undergo 6 types of genetic testing by scientific evidence, around 50% of respondents reported that they could not decide whether to undergo any type of genetic testing. 56.5% of them require ban on acquisition of unneeded personal information and only 39.7% of them are interested in ban on scientifically groundless tests.
Discussions: Our results suggest that members of the Japanese public have positive attitudes toward genetic testing, which provides useful information concerning the prevention and treatment of disease, and may attach less importance to scientific evidence supporting genetic testing than academic societies and the METI do.

PM20.06
Storage and future use of consumers' samples and data in direct-to-consumer genetic testing companies offering whole genome sequencing

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Whole genome sequencing (WGS) creates an unprecedented amount of data providing a powerful tool for clinical care and research. Recently, WGS has also been offered by commercial direct-to-consumer genetic testing (DTC GT) companies. The DTC offer of genetic tests has already brought attention to potentially problematic issues such as storage and future use of consumers' data and samples and informed consent. The aim of this study is to analyse the policies of DTC companies offering WGS with regard to the storage and future use of data and samples and how these issues are included in informed consent. The findings will be discussed in the context of relevant policy documents. Preliminary analysis of websites of two DTC GT companies, Illumina and GeneYouIn, reveals that they may, in fact, use consumers' samples and sequencing data for unspecified research. The companies specify that the genetic data of their consumers will be anonymized and that consumers have the possibility of opting out of research. Furthermore, the information about use of data and samples for research purposes is included in the consent forms, which consumers are asked to agree to or sign before undertaking the test. This study will contribute to the discussion on the ethical offer of DTC GT companies involving research activities. Part of this work is supported by an Erasmus Mundus Joint International Doctoral in Law, Science and Technology Fellowship, the Swedish Foundation for Humanities and Social Sciences and the CHIP ME COST Action IS1303.

PS20.07
Stigma: an ongoing challenge for families of individuals with Down syndrome

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Introduction: Current estimates suggest that over 6 million people in the world have Down syndrome (DS). Most individuals with DS spend the majority of their lives living with their family. Existing findings suggest that while some families have difficulty adapting to the ongoing challenges associated with raising an individual with DS, others adapt successfully and some even thrive. Stigma experiences may play a critical role in how well a family adapts. Therefore, the purpose of this study was to explore experiences with stigma among families of individuals with DS living in Ireland, Korea, Portugal, Thailand, United Kingdom, and USA.
Method: Over 1200 parents of individuals with DS completed an online survey which included a variety of self-report measures plus open-ended questions concerning how parents were informed of the DS diagnosis. A sub-set of parents were also interviewed.
Results: Many parents reported experiencing stigma when they were first informed of the diagnosis of DS. Stigma experiences also occurred when the individual with DS: 1) had serious health issues, 2) needed additional ser-

vices or resources, and 3) participated in activities outside the family home. Many parents felt professionals involved in health care and educational services often perpetuate the stigmas surrounding DS. Other factors thought to perpetuate stigmas were societal attitudes towards people with disabilities and national policies.

Conclusion: More research is needed to examine the link between stigma and adaptation in families of individuals with DS. Ideally this research should be cross-culture to capture variations both within and between different cultures.

PM20.08

Is there an ethical and/or legal obligation for healthcare providers to re-contact former patients in light of new genetic findings?

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Advances in genetics and genomics have the potential to allow more accurate diagnoses, improve knowledge of disease aetiology and risk, and inform therapeutic decisions.

For health benefits of genomics to be achieved, a number of issues in clinical implementation need to be addressed, including ethical and practice issues that have yet to be examined empirically and conceptually in any depth. Key among these is the clarification of professional responsibilities and obligations, as well as patient expectations, for re-contacting patients in light of new genetic findings and the re-interpretations of earlier findings. These issues are becoming more urgent with the arrival of Genomics England and related endeavors.

Our current three-year (2014-2017) ESRC funded project 'Mainstreaming Genetics: Re-contacting patients in a dynamic healthcare environment' http://ex.ac.uk/mgc addresses these questions. In particular it aims to:

1. Survey current clinical practices regarding re-contacting in the NHS and other European healthcare systems
2. Analyse the ethical and legal issues surrounding a potential responsibility or obligation to re-contact patients
3. Investigate patient and healthcare professional perspectives concerning re-contacting in different medical specialties using semi-structured interviews, vignettes, and questionnaires
4. Engage with stakeholders to integrate the above findings and analyses in the drafting of professional guidance or to develop a professional framework for making decisions about re-contacting patients

We will present and discuss the framing and initial findings of this project.

PS20.09

Quality of life of patients with the classic type of Ehlers-Danlos syndrome

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The Ehlers-Danlos syndrome (classic type, cEDS) is a non-inflammatory, heritable connective tissue disorder, caused by incorrect synthesis and structure of collagen. Patients with EDS present large spectrum of phenotypes, they may have cutaneous, musculoskeletal, cardiovascular anomalies and additionally non-localized pain of the whole body. Clinical symptoms of classic type of EDS have great impact on patients physical and mental health.

The purpose of the study was evaluation of the quality of life of patients with cEDS using Polish version of SF-36 questionnaire.

41 patients (37 women and 4 men) were enrolled in the investigated group. The SF-36 is organized into areas: physical health (PH), physical functioning (PF), role-physical (RP), bodily pain (BP), general health (GH), mental health (MH), vitality (VT), social functioning (SF), role-emotional (RE), general physical health (PCS) and mental health (MCS). All areas were evaluated. Additionally in investigation socio-demographic data (education), pedigree and clinical data (cEDS-positive relative in the family and strength of pain) were taken into account. Data analysis was carried out using the Statistical Package for the Social Sciences 21.0 (SPSS) (p-value≤0.05).

According to SF-36 test patients with cEDS have much worse than the average quality of life in areas: BP, GH and VT (physical functioning) and much better than the average quality of life in areas: SF, RE and MH (mental functioning).

Having at least one cEDS-positive relative decreased quality of life in SF, MH and MCS fields. Pain experienced by patients was the strongest factor decreasing physical and mental health quality of life.

PM20.10

Expanded carrier screening: are we ready? Results of an interview study with European geneticists

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Preconception carrier screening aims to identify couples in which both partners are unaffected carriers of the same recessive disorder and have a 25% risk of conceiving an affected child. Although carrier screening has traditionally been performed for a small number of common disorders, recent advances in genetics led to the emergence of expanded carrier screening (ECS) panels, which screen for an increasing number of recessive disorders at little or no additional cost. However, expansion of screening panels adds a new layer of complexity to carrier screening, making large-scale implementation of ECS controversial.

In-depth interviews were conducted with 16 geneticists from 8 EEA countries in order to explore participants' views on ECS. While they acknowledge the important potential benefits of ECS, most participants were reluctant to start actively offering screening to the general population. They raised concerns regarding limited understanding of genetics among laypersons, which may result in misinterpretation or confusion over complex issues of ECS and preclude informed reproductive decision-making. Furthermore, the absence of suitable infrastructure, limited public resources, and lack of expertise in genetic testing among healthcare providers were all identified as important challenges to ECS in the general population. However, most participants believed ECS should be made available to the couples who request it. In addition, some geneticists favored routinely offering ECS to patients undergoing artificial reproduction (AR). It was argued that providers of AR have an obligation to utilize state of the art technologies such as ECS to avoid conception of a fetus with a severe disorder.

PS20.11

Disclosing genetic information to kin: a quantitative survey to analyze ethical and practical issues

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When a person is diagnosed with a serious genetic anomaly, the disclosure of this information can be relevant for other family members when prevention measures or treatment exist that can improve prognosis and_in some cases_even prevent a death. Research on this question has rarely been explored from the standpoint of healthcare professionals. We work on an interdisciplinary research study dealing with healthcare professionals' practices and related ethical issues in France, where the legislative framework has recently evolved.

Thus, we initiated an online quantitative research survey to get a clearer picture of the challenges arising from this issue, its real-world consequences in terms of medical care-service practices, and the stances that frontline professionals have taken in response to this new legal framework. 204 responses were obtained. The findings highlight very different patterns of practices depending on the genetic diseases concerned, and on the professionals' competencies in genetics. 26% of respondents declare having no competency in genetics.

It is equally crucial to sharpen a number of points, such as the nature of genetic testing, their possible prescription by non-geneticist clinicians, the scope of genetic counselling and its role in the disclosure process, the nature of information to deliver to patients and their relatives. Are all the genetic diseases concerned by the disclosure of information to kin? What changes in medical practices does genetics imply? Does genetics require a change of responsibilities?

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PM20.12

How far do we need to go in transmitting genetic information to "family" members?

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Because of it hereditary nature, genetic information has a personal and family dimension. The diagnosis of a serious genetic anomaly in a patient can be clinically relevant for other family members when prevention measures or treatment exist. According to the French law there is a legal obligation for

patients to inform their relatives about this diagnosis if this information is relevant for their health. This information can be delivered directly by the patient, or indirectly through the specific genetic information procedure to family members. Thus, all relatives potentially concerned can access the genetic information if they want to.
In 2011, le legislator went further by allowing the communication of relevant genetic information to children born after a gamete or embryo donation. In this case, the prescribing doctor and the Center for Medically Assisted Procreation (CMAP) must be involved in this transmission if the donor allows it. The adjunction of this provision gives raise to the so-called "biological family" while ignoring the current French legislation on the secret of origins, on the governance issues devoted to the CMAP and on the consequences on the donor and on the child. We will present the ethical and legal issues (privacy, responsibility) rose by this procedure essentially based on "the right to know".
*This abstract is supported by the INCa project "Family disclosure in human genetics: Implications and implementation in case of familial genetic disorders" (subvention 2013-130) and the Canceropole Ile-de-France.

PS20.13
Oncogenetic and disclosure of information to kin ? Which ethical questions and practical issues ?

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In the context of disclosure genetic information to kin, the breast and ovarian cancer are largely present in the available littérature but from the standpoint of patients and relatives (Chivers-Seymour et al. , 2010 et Bradbury et al., 2012), and rarely, if ever, from the standpoint of healthcare professionals. In our view, it is necessary to explore also the interactions between healthcare professionals and patients during the actual genetic counselling process. So the contexte of oncogenetic in France, where a new legislation frame this subject, we propose to present some results issue from two distinct methodologies. The first are a quantitative research survey via a online questionnaire which was set up to get a clearer picture of the practice, to let emerge the bioethics topics and the difficulty in the practice of genetic counselling. We will present the results of questionnaire filled by professional working in oncogenetic field. The second is an ethnographic approach i.e. fieldwork with observation combined with interviews to be able to describe "ethics as a practice" (Fassin, 2008)). We therefore consider that the ethnographic approach is a complementary way to address the implementation of this new legislative provisions bound to be subject to interpretation by actors.
We propose to share our hypothesis that oncogenetic is a model for the application of disclosing information to kin and try to show the difference of approach in other genetic fields.
*This abstract is supported by the INCa project "Family disclosure in human genetics: Implications and implementation in case of familial genetic disorders" (subvention 2013-130) and the Canceropole Ile-de- France.

PM20.14
Straight from the lab to the market: General public engagement with Direct-to-consumer genetic testing

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With direct-to-consumer genetic testing (DTC), private companies provide tests and results directly to consumers in commercial transactions typically conducted online. DTC activities bypass traditional healthcare gatekeepers, representing a paradigm shift from medical to consumer and a transition from old to new economy regulatory and governance models. As consumer demand increases, concern has been raised about the potential for consumer detriment, especially psychological detriment, resulting from premature translation of genetic discoveries into for-profit tests.
When assessing the ethical, legal and social issues inherent in DTC, much of the focus has been on the interaction between individual consumers and DTC testing companies. The same holds true for assessing potential consumer detriment that drives consumer protection policy development and enforcement.
Modeling of the DTC space however has revealed morphing industry structures, increasing monetisation of DTC research databases and an expanding range of voluntary modes of consumer-to-consumer online engagement, illustrating the potential for consumer detriment extending far beyond an individual DTC transaction.
Fifteen hundred members of the Australian and American general public were surveyed to determine their ability to interpret and contextualize sample DTC genetic test results and the impact of individual risk interpretations on post-results psychological outcomes and behavioural intentions. Data was also collected on engagement with online sources of genetic information, online sharing of genetic information, interaction with healthcare professionals, and participation in DTC research. Results illustrate commonalities and differences by level of risk, disease, country and respondent demographic characteristics, providing insight to regulators and those developing governance frameworks.

PS20.15
Minors and their genetic identity

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The right to respect for privacy and family life encompasses both the right to have access to information regarding one's genetic origins as well as the right to have access to health information, including information about (the risk of) hereditary diseases. Regarding genetic testing, there is a universal consensus that minors have 'a right not to know'. This means that restraint must be exercised on genetic testing in order to safeguard a minor' right to an 'open future'. The right to respect for privacy and family life also requires that every child should be able to establish details of his biological origins, i.e. the 'right to know' who his genetic parents are.
Both situations - information regarding (the risk of) genetic diseases as well as knowledge about one's biological origins - concern a right to have access to information concerning one's genetic identity. However, the two basic principles applicable here are fundamentally different. The question is whether this different approach to the right to information about biological origins on the one hand and to genetic information about (the risk of) diseases on the other hand is justifiable?
In this study, we will examine the scope of the 'right to know' and the 'right not to know' in the light of the EVRM and the International Convention on the Rights of the Child. This study contains an overview of international literature and case law review regarding both rights in relation to genetic testing for health purposes and on personal origins.

PM20.16
What do patients think about the use of genome sequencing in the NHS?

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Abstract body: Genome sequencing is beginning to make its way into mainstream clinical practice in the UK, offering great potential for the effective diagnosis and future treatment of many conditions. As those most likely to benefit from the earliest uses of genome sequencing technologies, the views of patients and families affected by diagnosed and undiagnosed genetic conditions will be crucial in making decisions around its use in the context of clinical diagnosis and care. Through an online engagement project, comprised of videos, podcasts, questions and free text, we sought the views of these patients and families on the use of genome sequencing in the National Health Service (NHS) from the comfort of their own homes at a time convenient for them. Our results showed overwhelming enthusiasm from patients and families for genome sequencing to become a part of their routine care on the NHS and for sharing their genomic data for research. The findings also highlighted the value patients and families ascribe to the support offered by genetic counsellors. They cautioned though that at present they felt the NHS needed to do more to prepare for the integration of genome sequencing into clinical practice.

PS20.17
Assortative mating rate among deaf people in Yakutia (Eastern Siberia)

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Introduction of a sign language in special schools for deaf people led to growth of assortative marriages between them and contributed to increase of genetic fitness of deaf people. Combined effect of assortative mating and relaxed selection probably has doubled the GJB2 gene associated deafness in the US over the past 200 years (Nance et al., 2004; Arnos et al., 2008). High prevalence of the GJB2-deafness and relatively recent (~ 60 years ago) introduction of sign language among deaf people were recorded in Yakutia (Eastern Siberia). We performed study of the marital structure of deaf people in Yakutia as the first stage for following prediction of prevalence of the GJB2 gene associated deafness in Eastern Siberia. Data on marital status and family size of 121 hearing impaired individuals aged 25 to 67 years (mean 44.8 ± 9.3) from Yakutia was collected by special questionnaires. It was found that 114 of 121 respondents (94.2%) were married and/or had children, and 107 among married deaf individuals had a deaf marriage partner. Thus, the assortative mating rate among deaf people in Yakutia is 93.9%, that is one of the highest rates compared to other regions of the world (79.0% in the US; 46.8% in Turkey; 37.5% in Mongolia; 10.0-30.0% in Tunisia, and 10.0% in Varmland region of Sweden). The study was supported by the RFBR grants #15-04-04860_a, #14-04-01741_a, #15-44-05106-r_vostok_a, the State project #6.656.2014/K, and the Integration project of Siberian Branch of RAS №92.

PM20.18

Implementing a guideline to standardize the Citation Of BioResources in journal Articles (CoBRA) : a call to the scientific community.

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Background
Recently for the first time, a guideline called CoBRA -Citation of BioResources in journal Articles was proposed for reporting bioresource (biological samples, data and databases) use in research articles.
Methods
A standardized citation scheme for bioresources was developed as part of the 'Journal editors working subgroup' of the BRIF (Bioresource Research Impact Factor) initiative, in collaboration with scientists and science editors. Dissemination phase is supported by The EQUATOR (Enhancing the QUAlity and Transparency Of health Research) network.
Results
The proposed citation guideline was published (BMC Medicine, February 2015) with the following main features: each individual bioresource that is used to perform a study must be mentioned in the Method section and should be cited as an individual "reference [BIORESOURCE]" according to a delineated format using a unique identifier when possible. One way to acquire such an identifier is through the description of the resource in a meta-journal such as OJB (http://openbioresources.metajnl.com/).
Conclusions
Adopting the CoBRA scheme described here will improve the quality of bioresource reporting and will allow their visibility and traceability in scientific publications, thus increasing the recognition of bioresources' value and relevance to genetic research. The ESHG as well as other scientific and professional societies are being solicited to encourage their community to use this guideline.

PS20.19

A new regulation for a new era of genetic testing - revision of the Swiss Federal Act on Human Genetic Testing (HGTA)

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As genomic science advances, genetic testing is becoming more common, especially outside its usual application in the clinical context. New commercially available tests can provide hints on athletic talents, the appropriate individual diet or one's ethnical origins. The Swiss Federal Act on Human Genetic Testing (HGTA) does only regulate the clinical application of genetic testing. However, the new fields of application for genetic tests are at present not regulated. With the aim of preventing the misuse of genetic tests and therefore protecting human dignity and personality, especially of children, the HGTA is currently revised and the scope is broadened to all kind of genetic tests.
The preliminary draft of the HGTA envisages expanding its scope to genetic testing outside the medical context: Tests potentially harming persons in their dignity and personality must not be offered as direct to consumer tests (e.g. testing of children's athletic talents). Biological samples for genetic tests have to be collected by certified specialists (e.g. pharmacists). Only if there is no potential for misuse, tests may be offered directly to consumers. Testing on children outside a medical context is generally not allowed. The catalogue of criminal provisions is expanded to the misuse of genetic tests through private persons which should provide additional protection. Furthermore, it is planned to inform the public about genetic testing and its regulation.
The preliminary draft of the HGTA was on public consultation from February until May 2015. The revised HGTA is scheduled to enter into force at the earliest in 2018.

PM20.20

No more frontiers between research and care? Legal analysis of genome sequencing clinical implementation in France.

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Genetic information is specific. Unlike other kind of medical information, it is all at once personal, familial, predictive and potentially identifying. Due to these characteristics, the French legislator established a particular legal framework for this information either in the clinical setting or in research. The translation of new sequencing technologies in the clinical setting however questions the relevance of the different laws in force and gives rise to ethical and legal issues. The aim of this research is to address, in the light of the new dispositions adopted by the French law in 2011, the relevance of the legal framework related to genetic information in both research and clinic vis-à-vis the implementation of new sequencing technologies.
These technologies allow a large or the whole exploration of the genome. Thus massive information is produced and its interpretation may exceed the scope of the initial diagnosis. The possibility of incidental findings challenges the relevance of information and consent, the communication of results and the medical practitioner responsibility.
New technologies also blur the boundaries between research and care. The production of sequencing data in a clinical setting generates useful data for research. Conversely, relevant information for the patient may be produced in the course of genetic research. In this context, databases may be constructed and fed by both parties. The inputs of the various parties at stake are particularly relevant in the context of these new realities. Supported by FP7 3Gb-TEST GA 602269 and ESGI GA 262055.

PS20.21

Realising genomics in clinical practice: ethical policy making in action

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The translational gap between research and clinical applications is a significant deterrent to biomedical innovation and adverse regulation is sometimes cited as a disincentive for translating novel technologies. In this presentation, we report the findings of an 18 month PHG Foundation project which evaluated the ethical, legal and social implications of implementing next generation sequencing (NGS) (including NGS panel tests, whole exome and whole genome sequencing) into clinical care in the UK NHS. Our rationale was that the translation of these technologies into practice could be facilitated by addressing these ethical, legal and social challenges prospectively rather than on an ad-hoc basis as they arise. Five iterative stakeholder workshops were held on the following topics: the experiences of empirical researchers as NGS technologies are implemented in clinical settings; the impact of these technologies on the interface between research and clinical care in genomics; the influence of these technologies on the clinical patient pathway; using targeted approaches based on gene lists as a precursor to opening the genome and effect on consent, technical aspects, reanalysis and recontact; and finally the infrastructural prerequisites of evidence base development and mandated data sharing. Focusing on ethical challenges - obtaining a valid and informed consent, ensuring effective data sharing, developing managed systems for gene list construction, proportionate systems for reporting incidental findings, and the potential for reanalysis and recontact within a publicly funded but resource limited health system - has fostered a comprehensive framework for action, that has been welcomed by stakeholders and will build public trust.

PM20.22

Beyond personal: Deliberating the social, ethical and legal issues of personalised medicine in expert and citizen dialogues

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Today, the term personalised medicine is associated with different visions in biomedicine. Although it most often refers to biomarker-based strategies of patient stratification, it may also encompass proactive, predictive, and post-treatment approaches to healthcare. Personalised medicine thus is still a term subject to negotiation among stakeholders, including the general public. Additionally, technical, cost and data protection issues are up for debate. Since patients and citizens in general are called upon to make their biological tissue and genetic data available for personalised medicine-related research purposes, public participation is considered necessary to realise the vision of personalised medicine in socially robust ways on the EU and national level.

The paper presents the results of a deliberative project with experts and citizens on personalised medicine in the Austrian context throughout the year 2014. It analyses how experts from a variety of backgrounds (molecular biology, medicine, industry, ethics, and regulation) discussed the current framework conditions, expectations and challenges for personalised medicine in three stakeholder workshops, and compares these results with how members of the general public debated the term personalised medicine, prevention, cost, and data contribution issues in four citizen dialogue events. The paper focuses specifically on the central social, ethical and legal issues that were raised in these dialogue processes and reflects on the implications of these findings for communication activities directed towards the general public.

The project is funded by the Austrian Federal Ministry of Science, Research and Economy.

PS20.23

Are PGD users more liberal than policy makers? Attitudes of Israeli PGD consumers regarding justified uses for PGD compared with regulations

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PGD technology can increasingly detect genetic disorders and traits. Ethic committees are involved in understanding which usage for PGD is ethically justified. There has been considerable debate about the different uses of PGD and especially about the regulation of selecting embryos with non lethal, treatable or late onset conditions. Almost all guidelines worldwide ban PGD to less severe conditions or highly regulate it.

Exploring attitudes toward ethical and sociological aspects of PGD among 35 Israeli PGD users (carriers of autosomal recessive, dominant and X-linked disorders; HLA-matching) reveal totally different notions rather than these strict guidelines. High overall approval of PGD to any medical condition including non-lethal conditions, treatable conditions (e.g. deafness) and late-onset conditions (e.g. cancer predisposition) was mentioned and the onset age of condition did not play a role in the arguments for preventing any kind of abnormality. As to the controversial issue of social sex selection, the majority of surveyed subjects thought it would be appropriate to allow it for purposes of family balancing or cultural preferences. Yet, most of those in favor of this use thought performing PGD for this use only is an extreme step. In conclusion, our sample of Israeli subjects shows permissive attitudes to different application of PGD. These liberal attitudes of PGD users are not reflected in the guidelines made by the many ethic committees discussed these issues.

The isolation between medical-bioethics experts' decisions and PGD consumers' perspectives should not be ignored in the professional discussion and the design of policy making.

PM20.24

Ethical issues regarding presymptomatic and predictive genetic testing in Romania

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Genetic testing allow the identification of individuals who are carriers of some mutations in their genes, these mutations being the cause of some genetic disorders. Presently, three types of genetic testing are known: diagnostic, carrier and predictable. Predictive tests identify whether an individual is carrier of a genetic mutation and if there is the possibility to develop genetic disorders at a late on set. It is known that the first autosomal dominant genetic disease for which a predictive test was developed is Huntington's disease.

Unfortunately, if in the diagnostic and carrier tests we have the technical possibilities and they are correlated with the norms of the Romanian Medical System, when we talk about the predictive genetic tests, the situation is different because this type of diagnostic test isn't implemented yet in Romania. In Romania, we have some limitations regarding this type of predictive tests, because we don't have interdisciplinary teams in the hospitals to coordinate the management of these cases. Another limitation regards the ethical problems of information and patient consent. The lack of policy decisions, education campaigns, misinformation and lack of understanding regarding genetic diseases, leads to discriminatory practices.

All these problems need to be urgently solved and implemented in Romania, due to the special aspects belonging to them, especially in patients with Huntington disease.

PS20.25

Psychological Impact of Tumor Screening in *SDHX* Mutation Carriers Recruited in a 3 Year National Protocol

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Background: Tumor screening in hereditary paraganglioma allows early surgery. PGL-EVA is a 3 year French national protocol of tumor screening in SDHx mutation carriers.**Methods**: Depression (BDI-13), state and trait anxiety (STAI) and stress due to genetic test result (IES-R) were assessed at baseline in 197 subjects. Depression and state-anxiety were assessed again after tumor screening and then once a year.**Results**: At baseline, depression and stress were higher in index cases (p=0.023) than in relatives (p=0.013). After tumor screening, depression (p=0.003) and anxiety decreased (p=0.006). Final score of depression at 36 months (73 patients) was significantly associated with baseline score (p<0.001), without any significant change. Higher depression scores were observed in index cases vs relatives and in women vs men. Final anxiety scores were significantly associated with baseline scores (p<0.001), without any significant change.

In women, anxiety scores decreased between baseline and 36 months (p=0.03). The evolution of depression and anxiety scores was neither associated with the screening of new tumors, nor with the status of index cases vs relatives.

Anxiety score evolution was negatively associated with baseline trait-anxiety and stress.**Conclusions**: Tumor screening in genetically predisposed subjects does not lead to worsening emotional disturbances after three years of follow-up. A decrease of anxiety is observed in the more sensitive group of subjects at baseline.

PS20.27

Correlation between the levels of the awareness and the anxiety as factors, determining the quality of genetic counselling

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Introduction: This research is part of a longitudinal study of the factors that influence the GC process. We analyse how awareness and the basic attitudes toward GC can affect the ST. We have explored the effect of the diagnostic procedures, the maternity in advanced age, the family reproductive history and the impact of the ST.

Materials and Methods: We analyse the influence of the information about GC to the ST levels. The ST levels are explored with STAY. The awareness toward the GC is tested with authors' questionnaire. We tested women, pointed for GC after biochemical screening, amniocentesis and risk pregnancy. We assume that a higher level of awareness, in combination with the age as a risk factor, leads to increasing ST.

Results: The women with higher levels of awareness show higher ST. These levels show a direct relation with the awareness levels, in combination with the age as a risk factor and with the pathological reproductive history. The women with minimum knowledge about GC perceive it as serial procedure. They do not accept it as a risk. The ST levels of these women are lower and

no significant difference is mentioned toward their TA.
Conclusions: The awareness about GC, in combination with advanced age maternity, leads to an increase of the ST levels. Analysing this factor can help better understanding the GC process and suggests creating an algorithm for psychological support.

PM20.28
What IF? A European perspective on the use of WGS in the clinic

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The different perspectives in North America and Europe regarding the clinical use of whole genome sequencing (WGS) have been the subject of much debate. A number of areas of divergence, both across and within Europe and North America have been identified. Surveys of genetics professionals and other stakeholders seeking their perspectives on the issues raised have been carried out in North America and Canada but there is little data regarding the views of those with an interest in clinical genetics in Europe. In the context of 3Gb-TEST, a European coordination action, we have carried out a Europe-wide web based questionnaire of 147 individuals from 25 countries with a professional interest in whole genome sequencing to elicit their views on a range of ethical issues including opportunistic screening, consent, incidental findings (IF) and re-contact. There was large convergence for a longer consent procedure and returning clinically actionable IF, whereas other issues elicited divergent positions. The findings of this survey will be discussed along with the output of a European workshop to be held in May 2015 aimed at progressing the debate around these issues. Based on this, suggestions for further and more precise guideline formation for the transfer of WGS to the clinic will be outlined. Supported by FP7 GA 602269.

PUBLISHED ABSTRACTS

J01.01
MMP genes polymorphism and pregnancy loss

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Physiological pregnancy causes tissue reconstruction in maternal organism. Structural changes in developing embryo and forming placenta are even more complex and include not only cell division and connected tissue forming processes, but also cell migration, extra-embryonic tissue invasion into maternal tissues. All these processes demand intercellular space and tissue structure reconstruction, that depends on metalloproteinases and it's inhibitors system functioning. The data on a value of metalloproteinases genes allele variants in early embryogenesis disturbances are poor and controversial. The purpose of current study was to investigate the association between *MMP* genes polymorphism and pregnancy loss.
DNA samples were isolated from blood leukocytes of 134 women with early pregnancy loss and 144 women with normal pregnancy. -1607insG MMP-1 gene, A-8202G MMP-9 gene and C536T TIMP-1 gene polymorphisms were analyzed with allele-specific PCR. The distribution of genotype and allele frequencies of 1697insG MMP-1 gene polymorphism in women with early pregnancy loss was equal to control group. While percentage of homozygous genotypes of A-8202 allele of MMP-9 gene was increased compared to control group. Women with certain genotype had 2.6 fold spontaneous pregnancy loss relative risk increase. The distribution of genotypes of A-8202G MMP-9 gene polymorphism in women with early pregnancy loss differed from control group ($p<0.01$). There was no difference in genotype and allele frequencies of C536T TIMP-1 gene polymorphism between studied groups of women. Thus, the allele variants of MMP-9 gene are associated with pregnancy loss risk. This study was supported by the federal assignment № 6.98.2014/K from Russian Minist<ry of Science and Education.

J01.02
Three cases of rare *SRY*-negative 46,XX males with complete masculinization and a review of the literature

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Introduction: *SRY* (sex-determining region Y) gene located in Yp11.3 has been thought to trigger gonads to differentiate to testes and play a critical role in the sex determination process. To identify the clinical characteristics of *SRY*-negative male patients and detect candidate gene(s) in male sex reversal, we performed a retrospective study for cases of 46,XX males with a review of the literature.
Materials and Methods: *SRY*-negative 46,XX males who referred for cytogenetic analysis from 1983 to 2013 were studied for clinical findings, seminal analysis, basal hormone profiles, conventional cytogenetic analysis and polymerase chain reaction.
Results: Chromosome analysis for cultured peripheral blood cells of 8,386 individuals revealed 46,XX males in 19 cases (2.27%). Of these, three cases (0.04 %) were confirmed for the absence of the *SRY* gene.
Conclusions: We report three cases of rarely detected *SRY*-negative 46,XX males with data that will help to assess the incidence of sex reversal in Korea. Through a literature review of 102 cases, candidate gene(s) for sex determination were identified and include genes on autosomes or the X chromosome that may function to induce sex reversal under the absence of *SRY* with gene dosage variation.

J01.03
First results of fetal chromosome aneuploidy testing using cell-free DNA in Lithuania

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Backround. Circulating free DNA (fDNA) in blood of pregnant women is a mixture of genomic DNA of maternal and fetal origin. When applying the right method its analysis helps to detect fetal chromosomal aneuploydies with high sensivity and low fals positive rates.
Methods. Retrospective study of 80 singleton pregnancies between 9 and 20 weeks. Maternal blood was collected at Alfa Clinic (Vilnius) and send to Natera (San Carlos CA USA). cfDNA was isolated from maternal plasma, and targeted multiplex PCR amplification followed by sequencing of 19 488 po-

lymorphic loci covering chromosomes 13, 18, 21, X, and Y was performed. Sequencing data were analyzed using the NATUS algorithm that determines the copy number for each of the five chromosomes tested.
Results. Results were provided for 79 (98.8%) of the 80 cases. Most cases were in high risk pregnancy group (advanced maternal age and abnormal serum screening). Three cases were correctly identified as aneuploid, including trisomy 21 , trisomy 18 and rare case of mosaic case of X monosomy, with no false positive results. Test specificity showed 99% (CI 98-99.9 %) efficancy because all cases were confirmed by amniocentesis. One case was with low fetal fraction (2.3 %), however because of abnormal serum screening (very low PAPP-A and free beta-hCG) ammniocentesis and cyrtogenetic analysis was performed that revealed triploidy 69 XXY.
Conclusions. cfDNA testing in maternal blood using targeted sequencing of polymorphic loci at chromosomes 21, 18 and X showed accurate detection of fetal autosomal aneuploidies including chromosomal mosaicism. It helped to reduce the need of unnecessary invasive procuderes (CVS and amniocentesis).

J01.04
Assessment of possible correlation between sperm parameters and the incidence of aneuploidy in sperm of infertile males

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Introduction The causes of infertility are very complex, cytogenetic anomalies being one of the possible causes. In the last years was taken in consideration the hypothesis that not only somatic chromosomal anomalies but also germ cells chromosomal aberrations could lead to reproductive failure. The difficulties related to chromosomal evaluation in germ cells were overcome by the development of molecular cytogenetic techniques use.
Aim. We have study the hypothesis of a possible correlation between sperm parameters and the incidence of aneuploidy.
Materials and method In this study we used multicolor FISH probes for chromosome 13, 18, 21, X and Y and strict scoring criteria, in order to evaluate 35 patients with oligoasthenoteratozoospermia (OAT) and 20 individual with normal fertility.
Results The overall incidence of disomy in the OAT group showed a weak to moderate correlation with the semen parameters. In this study we found a moderate negative correlation between the disomy incidence and the sperm concentration (r=-0.49). By comparing the disomy incidence and the progressive motility and the normal morphology we found a weak negative correlation, the correlation coefficients were r=-0.39 and r=-0.36 respectively. Previous studies have reported negative correlation between the rate of chromosome aneuploidy and semen parameters.
Conclusions The molecular cytogenetic analysis allows the identification of patients with an increased risk for reproduction failure and facilitate an appropriate counseling in order to inform the patients about their reproductive options, the genetic preimplantation testing and the prenatal genetic tests that are available.

J01.05
Citogenetic and molecular genetic analysis of mirror duplication of chromosome 21

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INTRODUCTION: We present a new case of mirror duplication of chromosome discovered by cytogenetic analyses, and further studied with molecular markers. CASE RAPORT: Female newborn had all characteristics typical for Down syndrome: facial dysmorphia, muscle hypotonia, a single flexion furrow of the fifth finger on the right hand, short fingers and a heart anomaly. Derivative chromosome 21, was detected in all 30 studied metaphase cells. G banding technique showed translocation between two chromosomes 21, joined with terminal ends of the long arm. C banding technique showed a single centromere. QF – PCR analysis of newborn's DNA, showed six markers on the chromosome 21 with trisomic dialelic patterns (D21S1442, D21S1414, D21S1435, D21S1446, D21S1809, D21S1412), one marker with trialelic trisomic (D21S1437) and one normal heterozygous marker (D21S1411). Parents had normal karyotypes. After QF – PCR analysis from parents' DNA, normal markers ratios were found in father's DNA, but one marker (D21S1437) in mother's was in trialelic state . Mother had muscle hypotonia, a flat nasal bridge and a single flexion furrow of the fifth finger on the left hand, but she was a person of average intelligence and without other specific clinical characteristics. CONCLUSION: Literature data imply that region 21q22.3 is critical region for phenotype of Down syndrome, but this case also implies the role of region 21q21 (to wich marker D21S1437 maps) in some clinical features of Down syndrome.

J01.06
Association of folate, methionine cycle genes and the second phase of xenobiotic's detoxification genes in moldovian women with pregnancy losses

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Introduction: Pregnancy loss (PL) is a devastating reproductive problem affecting approximately 5% of women trying to conceive. The aim of this study is to provide a relatively comprehensive account of the association between mutations in GSTM1, GSTT1, GSTP1, MTHFR, MTR and MTRR and PL.
Materials and Methods: The prevalence of these polymorphisms was compared in 164 women with two or more pregnancy losses in the first and second trimester, and 64 women without history of miscarriages, with 2 healthy births. We used PCR and PCR-RFLP methods.
Results. The results of this study showed that women with PL have a higher proportion of the MTHFR 677TT (OR=3.35), MTHFR 677CT (OR=1.16), MTR 2756AG (OR=1.21) and GSTT1 null genotype (OR=1.09) compared with the control group, however, the difference was not statistically significant. The prevalence of GSTT1 null+MTHFR 677CT and compound 677CT/1298AC were higher in the study group than in the control group (14% versus 8% and 27% versus 23%, respectively). The gene polymorphisms MTHFR 677TT, MTR 2756GG were significantly more frequent in the patients with second trimester PL (P<0.05) and the prevalence of GSTM1 null, GSTP1 105 Val/Val, MTRR 66AA were more higher in the patients with first trimester losses, but there was not statistically significant.
Conclusion: The risk of PL is higher for women carrying MTHFR 677TT, MTHFR 677CT, MTR 2756AG and GSTT1 null. MTHFR 677TT and MTR 2756GG genotypes were significantly more frequent in the patients with second trimester PL (P=0.006. OR=0.16 and P=0.002. OR=0.06).

J01.07
Genetic causes of male infertility in patients with azoospermia from Serbia - eight years experience

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Introduction: According to the World Health Organization, infertility affects 10-15% of couples and male factor represents 30-50% of cases. Genetic factors were found to have important roles in the etiology of idiopathic azoospermia and severe oligospermia patients, affecting sperm production or sperm transport.
Materials and Methods: Since 2006, samples of 62 azoospermia patients were analyzed using cytogenetic, molecular cytogenetic and molecular genetic methods. Cytogenetic analysis were performed on cultured PHA-stimulated blood lymphocites using GTG banding. Fluorescence in situ hybridization (FISH) was performed using a Y chromosome specific DYZ3 locus. Presence of microdeletions in AZF region on Y chromosome was analyzed using multiplex PCR reactions for amplification of ZFX/ZFY, SRY, AZFa, AZFb and AZFc regions.
Results: Cytogenetic analysis showed presence of aberrant karyotype in 9 patients. Structural chromosomal aberrations were detected in four patients (one with dicentric Y chromosome, two with deletion of long arm of Y chromosome, delYq11.22 and one patient with balanced translocation). Numerical chromosomal aberrations were detected in five patients (four of them with 47,XXY karyotype and one patient with two Y chromosomes-47-,XYY). Results of molecular genetic testing showed that five patiens had AZF deletions (two with AZFc deletion and three with AZFbc deletion). All patients were offered genetic counseling service in our hospital.
Conclusion: The results of this study have determinated the cause of azoospermia in 19.35% of all analysed patients. Given that our results showed high prevalence of genetic aberrations in infertile men, application of genetic testing in such cases is fully justified.

J01.08
GSTM1 and GSTT1 null genotypes and the risk of non-obstructive azoospermia: a case-control study in an Iranian population

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Approximately 15-20% couples are suffered from infertility and about half of these cases are due to male infertility. Spermatogenesis impairment is the most common cause of male infertility. Reactive oxygen species (ROS), such as the superoxide anion and hydroxyl radical, can be produced by human spermatozoa. There is growing evidence that oxidative damage to the human spermatozoa membrane is an important pathophysiological mechanism in human male infertility. The aim of the present study was to investigate whether there is a genetic association between the glutathione-S-transferase (GST) enzyme and idiopathic male fertility. We examined GSTT1 and GSTM1 polymorphisms in 100 infertile men with non-obstructive azoospermia and 100 fertile men as the control group using multiplex PCR. The GSTM1 null genotype was present at frequencies of 0.57 in infertile cases and 0.42 in controls (OR=1.83, 95%CI=1.04-3.20; P=0.034).These frequencies were 0.15 and 0.15 for GSTT1 null genotype respectively (OR=1, 95% CI=0.46-2.17; P>0.05).The frequency for the null genotypes of both GSTM1 and GSTT1 in patients was 72% versus 57% in controls (OR=1.93, 95%CI=1.07-3.49; P=0.027). Our results suggest an association between GSTM1 null genotype and idiopathic azoospermia.

J01.09
A double translocation found in prenatal diagnosis

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Translocations are the most frequent structural chromosomal abnormalities and may pass undetected through generations. Miscarriages, infertility or the birth of a child with an unbalanced form of translocation usually reveals the existence of a familial chromosomal translocation. The development of new techniques, such as array Comparative Genomic Hybridization (aCGH), has increased the resolution of novel or rare microdeletions/microduplications, but chromosome analysis remains the gold standard to delineate chromosomal structural rearrangements.
The authors present a case of a 45-year-old pregnant woman referred for the first time to our prenatal center. She had a previous history of ten miscarriages, not investigated, and five healthy children. Amniotic fluid (two cultures) and parent's blood cultures were performed according to the protocols established in the laboratory. Oligonucleotide aCGH was applied.
Cytogenetic analysis of amniotic fluid revealed a double translocation: t(1;2)(p34.1;p23)mat and t(1;5)(q10;q10), a *de novo* situation. aCGH analysis didn´t detected any gain or loss involving the translocated segments and the ultrasound parameters were normal. The couple decided to continue the pregnancy.
Cases of unrelated double translocations are extremely rare, there are only seven cases described and six of them were *de novo*. The presence of an inherited and a new translocation detected in a prenatal diagnosis implies a more careful approach in the pregnancy follow up. Currently prenatal diagnosis can benefit from the advances of the molecular techniques that are very useful for the precise characterization of chromosomal anomalies.
The authors will present a review of the previous described cases and compared with this case.

J01.10
The most frequent mutation in the CFTR gene among CF children and adult CF patients

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Cystic fibrosis is an autosomal disorder with high clinical variability that is associated with CFTR mutations.
In this work we analyzed DNA CF children and CF adult patients from different regions of Russia to compare the distribution of the 19 mutations (CFTRdele2,3, F508del, I507del, 1677delTA, 2143delT, 2184insA, 394delTT, 3821delT, L138ins, 604insA, 3944delTG, G542X, W1282X, N1303K, R334W, 3849+10kbC>T, S1196X, 621+1g>t, E92K), that are the most frequent mutations in Russian Federation.
We analyzed 296 CF children and 501 adults CF patients. The middle age of adult patients is 27 years old and the middle age of children is 7 years old.
The most frequent mutations (F508del and CFTRdele 2.3) were found among children F508del - 53.2% and among adult patients 50.6%, CFTRdele2.3- 6.7% among children and 6.5% among adults and didn't discover the significant distinctions between groups. Mutations I507del, 2143delT, 2184insA, 394delTT, 3821delT, L138ins, 604insA, 3944delTG, G542X, N1303K, S1196X, 621+1g>t, E92K were met from 0 to 5.4% and also didn't find the significant distinction between groups. For mutations W1282X, 1677delTA, 3849+10kbC>T, R334W was found differences among groups ($p < 0.05$). Mutations W1282X and 1677delTA are associated with heavy clinical phenotype and in adult CF patients was found less often than among CF children. Mutations 3849+10kbC>T and R334W belong to IV class of mutations in CFTR gene and as result is a mild form of CF. In adult CF patients was found more often than among CF children.
Thus in our work we can see that "mild" mutations are found more often among adult CF patients and "severe" mutations are among children.

J01.11
Restrictions of the QF-PCR method in detecting fetal aneuploidies

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Quantitavive fluorescent polymerase chain reaction (QF-PCR) has proved to be an accurate, robust and efficient method for detecting the most common aneuploidies.
We reported some restrictions in testing of prenatal samples using commercial QF-PCR assay.
At 33 weeks of pregnancy, amniocentesis and QF-PCR was performed because of ultrasound abnormalities. In the absence of the bloodstaining the allele ratios were inconclusive showing two different profiles. After QF-PCR of mother blood sample was performed, we found out that this result was consistent with mother's profile.
In our second case, a 38-year old female underwent chorionic villus sampling due to advanced maternal age. Conventional chromosomal analysis showed 47,XXY[6]/47,[6]/47,XY,+8[19]/47,XY,+18[35]. A subsequent QF-PCR after amniocentesis revealed a normal 13,18,21 chromosomes pattern and the abnormal sex chromosome profile which could be compatible with a XY/XXY mosaicism. Cells with trisomy 18 were probably confined to the placenta or presented in less than 10% of amniocytes.
In another case, a CVS was obtained from pregnant woman with fetal ultrasound abnormalities. The QF-PCR of multiple X/Y specific markers showed abnormal sex chromosome pattern. AMXY marker showed a skewed 1.7:1 ratio, Yq marker did not amplify. After karyotyping, FISH and chromosome Y microdeletion analysis, the results showed 46,X,del(Y)(q12).ish der(Y)(wcpY+,DXYS153+,SRY+,DYZ3+,DXYS153+). In cases of abnormal markers for sex chromosomes further testing is recommended.
The presented cases demonstrate that caution should be taken when conflicting results are observed or abnormal X/Y pattern is present in prenatal sample. Analysis of prenatal samples might be complicated by variable sample quality, mosaicism or maternal cell contamination.

J01.12
Prenatal finding of pericentromeric inversion of chromosome 3

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Every prenatal finding of not clearly pathogenic chromosomal abnormality is a very difficult situation in genetic counselling. Even postnatally, it is frequently impossible to certainly correlate the cytogenetic finding and particular phenotypic abnormality.
We present a case of Czech patient with prenatal finding of de novo pericentric inversion 46,XX,inv(3)(p11.2q21) in the amniotic cells obtained by an amniocentesis performed for the advance maternal age, there were no other risk indicators.
In literature, there is only one similar case in Spedicato FS et al.: Pericentric inversion inv(3)(p11q21). [J Med Genet. 1984 Oct;21(5):396.] The inversion was detected in a newborn with a prominent forehead and receding chin, a left preauricular tag, a long philtrum, and pendulous cheeks. Its familial occurrence in other normal relatives (father, brother, paternal aunt and her daughter) emerged afterwards. Today in our case we had the result of array-CGH in addition - no deletion was detected in the breakpoint or anywhere else.
With this knowledge parents in decided not to terminate their pregnancy (with acceptable residual risk which is always inevitable). The rest of pregnancy proceeds without any complication so far - the estimated date of delivery is in the middle of February. After the delivery, the child will be examined and the prenatal cytogenetic finding will be confirmed (and extended) by the analysis of the peripheral blood sample. All of these results will be presented on the meeting.
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J01.13

Analyzing The FSHR Polymorphisms in İnfertile Turkish Woman

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Follicle-stimulating hormone (FSH) by binding to a specific receptor play a major role in the development of follicles and regulation of steroidogenesis in the ovary, and spermatogenesis in the testis. There were studies analyzing the effects of the SNP in exon 10 (codon 307 and 680) and in the core promoter region (at position -29) of the FSHR gene on spermatogenesis, but for our knowledge there were no studies analyzing the effects of these 3 SNPs' combinations on female fertility.

In this study, the allelic, genotype, and haplotype frequency distributions of these 3 SNPs in the FSHR gene were analysed 102 infertile women, 99 unrelated healthy control individuals. The distribution of the polymorphisms was confirmed by Hardy-Weinberg equilibrium test. In infertile patients, linkage disequilibrium between the -29, Ala307Thr and Asn680Ser of FSHR polymorphisms was indicating same linkage disequilibrium. However, there were no statistical differences ($p>0.05$) in the allele, genotype and haplotype frequencies of the polymorphisms between the infertile patients and the controls. Also there was no differences between FSH, LH and E2 levels and genotypes in -29, 680 SNPs ($p>0.05$). It was found only significant relation between 307 SNP GG genotype and FSH level ≤10 and GA genotype and FSH level >10 ($P=0.016$).

The present study was the first to determine the polymorphism of the FSHR core promoter at position -29 alone and in combination with the two common SNPs in exon 10 in Turkish infertile women population. These findings indicate the significance of Ala307Thr GA genotype may be a predictive marker for poor ovarian reserve.

J01.14

Maternal plasma expression of fetal hsa-miR-99a in pregnancies with congenital heart defects

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Introduction: Only one out of eight congenital heart defects (CHD) is associated with chromosomal disorders, yet non-invasive prenatal diagnostics focuses majorly on screening for trisomies instead of cardiac malformations. Assumed to modify up to 60 percent of human protein-encoding genes, microRNAs play an important role in cardiogenesis and present an intriguing marker for standalone CHD-screening. The aim of this study was to investigate the role and the maternal plasma expression of fetal hsa-miR-99a in pregnancies affected by CHD.

Materials and methods: Peripheral blood samples were collected from 39 pregnant women: 22 pregnancies were affected by CHD and 17 pregnancies were CHD-free controls. MiRNA was isolated from maternal serum and quantitative real-time PCR was carried out to determine the expression of total-miRNA, u6-snRNA and hsa-miR-99a.

Results: While there was no significant difference between the miRNA concentrations among CHD-affected and control group (5.54 ng/µl vs. 6.40 ng/µl), we found significantly up-regulated hsa-miR-99a levels in the CHD-affected group (1.78×10^{-2} ng/µl ± 3.53×10^{-2} vs. 1.09×10^{-2} ± 3.55×10^{-3} ng/µl, $p = 0.038$).

Conclusions: According to our study, hsa-miR-99a is overexpressed in pregnancies affected by a variety of CHD, could be used to monitor fetal cardiogenesis during early pregnancy and to non-invasively screen for CHD independent of chromosomal disorders.

J01.15

Study of the FMR1 gene structure among women with ovarian dysfunction from the Basque Country

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Primary ovarian insufficiency (POI) is defined as irregular menses and elevated gonadotrophin levels before or at the age of 40 years. The FMR1 intermediate (35-54 CGGs) and premutation (55-200 CGGs) alleles have been related with the development of this condition. A group of 68 women with ovarian dysfunction of unknown aetiology and 47 control women from the Basque Country was analyzed. The length of the repeat and the AGG interspersion pattern were evaluated. In relation to the number of CGG repeats, the frequency of alleles with 35-200 CGG repeats was statistically higher in the patient group (12.50% vs. 0%). This group was divided into three categories: group 1, irregular cycles, reduced fecundity and FSH levels <10 IU/l; group 2, irregular cycles reduced fecundity and FSH levels ≥10 IU/l and group 3, amenorrhea for at least 4 consecutive months and FSH levels ≥10 IU/l. In the three subgroups the frequency of alleles with 35-200 CGG was statistically higher than in controls. Regarding the AGG interspersion pattern, many of the intermediate and premutation alleles among patients appeared to have two interruptions (70%), the first AGG located in the 10th position (75%) and more than 15 CGG at the 3' end (83.3%). Interestingly, among these alleles the predominant structure was 9+9+n, indicating a loss of AGG interruptions at the 3' end. Therefore, the data showed that among patients the alleles were more unstable and that this instability influencing the FMR1 expansion might be related with the development of an ovarian dysfunction.

J01.16

The improvement of prenatal screening of aneuploidies and preeclampsia in Ist to IIIrd trimester by 1T QUAD test

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The aim of the study was to verify the screening success of 1T QUAD test (PAPPP-A, fbhCG, AFP, PlGF) and examination of decerased PlGF levels within IInd-IIIrd trimester.

Biomarker levels were examined on Delfia Xpress and LifeCycle 3 and Preeclampsia Predictor software were used (Perkin Elmer). MAP, arteria uterina pulsatility index and extended genetic risks questionnaire were collected.

Efficiency of PAPP-A, fbhCG and 1T QUAD was compared in 400 pregnancies, retrospective evaluation of PlGF and PAPP-A in 35 pregnancies with PE.

The 1T QUAD has significantly higher success than PAPP-A and fbhCG only as reliability for aneuploidy and PE risk calculation. Trisomy 21 risk 1:50000-1:100000 was in 56% in 1T QUAD compared to "dual" strategy with 2.1%. It provides optimal NIPT indication according selected risk criteria. Same PE screening success is guaranteed by decreased PlGF levels in IInd trimester as in Ist one, in intermediate and late PE detection even in negative Ist trimester PlGF screening. PlGF 5th percentile levels were created for 9-38th week for improvement of all PE types prediction. PlGF levels higher than 270 pg/ml in IInd and IIIrd trimester eliminate in 100% PE risk.

The 1T QUAD strategy significantly improves aneuploidy and PE detection in Ist trimester. Decreased PlGF levels in IInd trimester within 14-20th and 20-29th weeks significantly improve detection of not only aneuploidy and all types of PE but also severe disorders of prenatal development.

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J01.17

The Role of ADAMTS5 Proteases In Male Infertility

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Introduction: The defect of matrix remodeling and the imbalance of ECM's construction and destruction are important in male infertility. It is not enough to explain idiopatic cases because there are lots of unknown subjects in studyings which make clear spermatogenesis process.

The development of molecular methods is needed because it lets us to understand the fertilization steps and will be used in these patients. Lately there are lots of studies in literature about metalloproteinases which have different physiological functions in human metabolism and play a crucial role in construction and destruction of ECM. But there are very few studies about male infertility

İn this study, our aim is to investigate the role of ADAMTS5 proteases which is responsible for degrafdation of ECM in spermatogenesis.

Material method: In this study, we divided infertile mens into four groups. We isolated protein from semen samples taken from 10 control, 10 oligospermia, 10 idiopatic infertile and 5 azoospermia groups. We analysed protein levels with western blot.

Results : According to results, we determined that the protein levels of ADAMTS5 decreased 2,68 and 1,66 fold in azospermia and oligospermia groups respectively than control groups. And we didn't find any differences between control groups and idiopatic infertile groups.

Conclusion: It is very important to understand molecular function and organization of ADAMTSs which will be significant in enlightining the process of spermatogenesis in male infertility. Therefore we need to determine more biomarker related to male infertility.

J01.18

Major congenital malformations and neonatal complications in a cohort of Spanish children born after assisted reproductive techniques and associated factors

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Introduction
More than 4 million infants have been born after assisted reproductive techniques (ART) around the world. There are contradictories data about neonatal outcomes and risk of major congenital malformations (MCM) in this population. Objective: to describe the prevalence of neonatal complications and MCM at the age of one year in a cohort of children born after ART compared to spontaneous births, and to determine associated factors.
Material and methods
Prospective cohort study of children born from women undergoing IVF/ICSI treatment between may 2012-february 2013, in Assisted Reproduction Service of Spanish tertiary Hospital. Cause of infertility, embryo transfer (ET) day (D), maternal age, neonatal outcome and presence of MCM were analysed.
Results
107/121 live births were assessed (participation 88%). Mean maternal age: 32 years. 26% were multiple gestations (MG). Prematurity in 22/107 (20%) associated to MG (19/22). Mean birth weight: 2900 g (p40). 19/107 needed hospitalisation. At the age of one, 11 presented MCM (10%) according to ICD-10 classification: congenital malformation of cardiac septa (6), aortic valve (1), nervous system (2) and congenital hydronephrosis (1). It was associated significantly ($p<0.05$) to ET D5/D6 and feminine infertility was a protective factor. No association with maternal age or MG.
Conclusions
Neonatal hospitalisation and prematurity were higher than in spontaneous births, but it seems to be associated to MG. Prevalence of MCM was higher than expected and congenital heart disease was the most frequent. The association with late ET suggests that the culture might be a predisposing factor. Further studies will be necessary to assess these results.

J01.19

The Association Between Inherited Thrombophilia and Spontaneous Abortion: A Comparative Study

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Purpose
To evaluate the association of trombophilic factors and spontaneous abortion we studied the genotypes of Factor V Leiden (FVL), Protrombin G20210A, MTHFR C677T, PAI-1 4G/5G, ACE I/D, eNOS E298D, Apo E E2/E3/E4 for spontaneously aborted fetal materials, their mothers and fertile women as control group.
Methods
Target SNPs for each gene were analysed by real time PCR technique after genomic DNA isolation from maternal blood-EDTA, control group blood-EDTA and spontaneously aborted fetal tissues. Statistical analysis was done with medcalc statistical programme. Twenty-three spontaneously aborted materials, twenty-two mothers who had these abortions and twenty-two fertile women were included in this study.
Results
PAI-1 4G/5G+4G/4G ($p=0.0017$), 4G/4G ($p=0.0253$), eNOS 894GT+894TT ($p=0.0011$) genotypes and T allele ($p=0.0185$), Apo E E3/E4+E3/E2+E2/E4 ($p<0.0001$) genotypes, E2 ($p<0.0001$) and E4 ($p<0.0001$) alleles were higher in spontaneously aborted fetal materials comparing with their mothers and control group. Factor V Leiden, Prothrombin G20210A, MTHFR C677T, ACE I/D polymorphisms were not different in aborted materials and both mothers and control group ($p>0.05$).
Conclusion
PAI-1 4G/5G, eNOS E298D and Apo E E2/E3/E4 polymorphisms were associated with increased risk of spontaneous abortion comparing the abortion materials with their mothers and control group.

J01.20

The analysis of polymorphism of genes cyitokines at women with chronic salpingoophoritis

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The chronic salpingoophoritis(CSO) - a widespread infectious inflammatory disease of appendages of a uterus with a relapsing course. Search of associations of polymorphic loci of genes of TNFA, IL1B and IL1RN with development of CSO was a research objective. Comparison of distribution of frequencies of genotypes and alleles of a polymorphic locus 3539C>T gene IL1B between groups of patients and control revealed authentically significant prevalence of a genotype of IL1B*-3539C/C in group of control (P=0,0045). This genotype can be a marker of the lowered risk of development of a chronic salpingoophoritis for which odds ratio of chances made 0,14. As a result of the carried-out analysis of a polymorphic locus 3539C>Tgene of IL1B was revealed association allele by IL1B*3539T with the increased risk of development of a disease in the studied group (OR=3,03).

J01.21

Hereditary thrombophilia as one of the causes of pregnancy losses in Moldavian women

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Introduction: Recurrent pregnancy losses (RPL) are affecting approximately 3% of women and are a significant clinical problem, in many cases associated with thrombophilia. An increased risk of thrombophilic complications occurs during pregnancy, owing to reorganization of the coagulation/anti-coagulation and phibrinolitic organisms systems that are thought to be an evolution adaptation to reduce blood losses during delivery.
The aim of this study was to screen the incidence of five thrombophilic mutations and to determine if their presence is associated with RPL.
Materials and Methods: using RFLP/PCR were screened the FII(G20210A), FV(G1691A), VKORC1(C1173T and G1639A) and PAI1(4G/5G) mutations in case/control groups. Case group contained 274 women with 2 or more abortions and control group - 68 women with at least 2 normal pregnancies and no abortion. For data analysis was used Chi-square test and Odds ratio (OR) calculations.
Results: the incidence of homozygous and heterozygous mutations in FII (OR 2.18, 95% CI 0.26, 18.13, $p>0.05$), FV (OR 6.86, 95% CI 0.90, 52.48, $p<0.05$) genes was associated with RPL in the case group and represent a risk factors. The same is true for homozygous mutation (T1173T) in the VKORC1 gene (OR 3.26, 95% CI 1.07, 9.91, $p>0.05$). No associations with RPL were obtained for mutations in PAI1 gene.
Conclusions: obtained data demonstrates that mutations in FII, FV and VKORC1 genes may play a crucial role in case of RPL. Is necessary to perform the screening for genes mutations causing hereditary thrombophilia in women with RPL and apply/adjust the anti-coagulation treatment.

J01.22

Preimplantation genetic diagnosis (PGD) for carriers of balanced translocations

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With the help of modern in vitro fertilization technologies (IVF) it is possible to identify and transfer to uterus only genetically normal embryos from couples where one of the partners is a carrier of chromosomal anomaly. Such patients have high risk of producing genetically compromised embryos[1].
Of 236 IVF+PGD cycles, 30 (335 embryos) were held for rearrangement carriers to identify embryos with balanced karyotype by FISH. The investigation was made on single blastomere, which was biopsied on the cleavage stage.
Among 335 embryos analyzed, 25,97% were balanced but only 14,03% had euploid set of chromosomes. Analysis of embryo karyotype according to the translocation type revealed that balanced euploid specimen occur in 2 times

more often in case of Robertsonian translocation than for reciprocal ones (namely 2,3 and 1,2 embryos per cycle, respectively). Nevertheless, total proportion of balanced samples for 2 types of structural rearrangement was approximately equal. Results of the diagnostics according to the sex of translocation carrier showed that when woman had the balanced rearrangement 29,41% of embryos were balanced while in case of paternal carriership the corresponding percentage was slightly lower (24,07%). Obtained data is consistent with the results of other researchers [2,3].
Modern genetic technologies give a chance for patients with structural rearrangements to avoid gamete donation and have healthy genetically related progeny.
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J01.23
Association between Folate Pathway Genes and Cleft Lip With or Without Cleft Palate in a Chinese Population

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Objective Non-syndromic cleft lip with or without cleft palate (NSCL/P) is a common congenital defect and gene-environmental factors involve in this disorder. Periconceptional intake of folate may reduce the risk of NSCL/P. The present study was designed to investigate if genetic variations in three folate pathway genes, including transcobalamin II (TCN2), methionine synthase (MTR) and betaine-homocysteine methyltransferase (BHMT), contribute to NSCL/P aetiology.
Methods DNA was obtained from 481 individuals with NSCL/P and 558 healthy subjects. Three known single nucleotide polymorphisms (SNPs) (rs1801198, rs955516 and rs3733890) present in the TCN2, MTR, and BHMT genes were genotyped by Matrix-Assisted Laser Desorption/ Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) approach.
Results SNP rs955516 showed allelic association with NSCL/P, higher frequencies of A allele in NSCL/P patients compared with those healthy individuals (X2=11.200, P=0.0008). Higher proportion of NSCL/P patients carry rs955516 AA and rs3733890 AA genotypes (OR=1.84, 95%CI=1.29-2.63; OR=1.65, 95%CI=1.08-2.53). The gene-gene interaction test show evidence of trans-phase interaction for MTR and BHMT combinations (X2=20.320, df=6, P=0.0024).
Conclusion Our study suggests that gene-gene interaction of MTR and BHMT genes play a vital role in the pathogenesis of NSCL/P in Chinese population.

J01.24
Prenatal diagnosis of cryptic translocation t(5p;17q) detected with fluorescent in situ hybridisation

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Reciprocal chromosomal rearrangements, de novo or inherited, often raise a concern about the fetal health and outcome in the affected pregnancies. The size and origin of the translocated chromosomes could be variable. Cryptic translocations often remain undetected and misdiagnosed. Several studies confirmed that there could be a loss of a certain amount of genomic material within breakpoints, which lead to uncertainties in predicting the pregnancy outcome.
A 38 year pregnant woman approached our clinic for a genetic counseling. This was her first pregnancy, ultrasound follow up showed normal fetal growth; the amniocentesis was done due to the advanced maternal age. Conventional cytogenetic report showed putative deletion of short arm of chromosome 5. Additional cordocentesis has been done at 18 week of pregnancy. MLPA analysis showed that 5p critical region was present. FISH has been done, using 5p/q probe (Cytocell aquarius, Cat No LPU 013), which showed cryptic de novo translocation 46,XX, ish t(5p;17q). Reduced fetal growth was recorded after 20th week, and together with cytogetetic finding were decisive for the parents to terminate the pregnancy. Examination at autopsy showed dismorphism consistent with cri du chat syndrome-micrognathia, hypertelorism, SGA, as well as underdeveloped brain for gestational period. Novel technologies in molecular cytogenetics and array techniques could help in detecting minor imbalances and decrease the risk of the birth of malformed fetus. Combination of several prenatal methods (ultrasound and genetic techniques) can help in decision making concerning prenatal diagnosis.

J01.25
The correlation between sperm DNA fragmentation and IVF outcome in Iranian population

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Background: as previous studies have mentioned that sperm chromatin integrity is vital for successful pregnancy and implantation. This study was designed to examine the relationship between the results of sperm chromatin structure assay (SCSA) and the outcome of IVF.
Method: After collection of semen samples from men of proven fertility (n = 47) and patients from an infertile population (n = 66), semen specimen were frozen at 80°Cfor later SCSA analysis. On the day of analysis, the samples were quickly thawed and analysed immediately. Spermatozoa are treated with a low pH buffer that denatures DNA at sites of DNA strand breaks. The sample is then stained with fluorescent intercalating dye, Acridine orange. Flowcytometry analysis was used to separate ssDNA as fragmented DNA from dsDNA as integrated DNA reported as DFI (DNA Fragmentation Index). Current references indicate that DFI more than 27% correlates with IVF failure.
Results: In this study significant relationship between DFI over 27% of couples with IVF failure (43.3%) compared to normal group (13.3%) was observed ($p<0.05$).
Discussion:The above analyses show that the SCSA infertility test is significantly predictive
for reduced pregnancy success using in vivo, IVF. These data clearly indicate that the SCSA is an important component of the infertility workup and suggest that if a man has a DFI of >30% that IVF should probably not be considered.

J01.26
Ring chromosome 22 in azoospermic patient: case report

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We report on a patient with rare genetic abnormality, ring chromosome 22. The patient is 30-years-old infertile man who presented proportional normal stature (tall 178 cm, weight 90 kg), normal masculinization and no sign of congenital abnormalities, chronicle diseases and mental retardation. The proband had fertile 28-years-old sister, which had healthy daughter. Non-obstructive azoospermia was diagnosed by repeated sperm examination in patient, but detailed analysis of ejaculate sediment allowed to find single abnormal morphology spermatozoa and ~200 immature germ cells.
Chromosome analysis was performed on cultivated peripheral blood lymphocytes using GTG- and C- staining techniques. FISH analysis with subtelomeric chromosome 22 probe (Tel Vysion 22q, Abbott Molecular) was done on metaphase chromosomes and interphase nuclei of peripheral lymphocytes. Y chromosome microdeletions were analyzed by multiplex PCR for SRY, ZFX/ZFY and 20 Yq specific STS loci from AZF a,b,c regions.
Chromosome analysis showed 46,XY,r(22)(p11q13) karyotype. FISH analysis confirmed a presence of non-mosaic ring chromosome 22, at that the breakpoints were localized in telomeric 22q region. Since no chromosomal material was loss, revealed chromosome abnormality is balanced. No Y chromosome microdeletion was detected by PCR analysis. Karyological analysis of immature germ cells from ejaculate sediment showed incomplete spermatogenesis arrest at prepachytene and pachytene stages of prophase I of meiosis. Evidently, it was resulted from a presence of ring chromosome in the karyotype.

J01.27
MED12 gene mutations and leiomyoma: A report from Iran

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Background: Uterine leiomyomas are the most common benign tumor arise from smooth muscle tissue in reproductive-age women which cause reduced fertility rates. These tumors are the most common cause of hysterectomy and surgery and seriously affect women community's health. Recent identification of MED12 (the mediator complex subunit 12 gene) mutations in most uterine leiomyomas provides a new and promising candidate gene for understanding the cause and tumorigenesis of leiomyomas. The aim of this study was to investigate the frequency of MED12 intron 1 and exon 2 mutations in uterine leiomyomas of Iranian patients.
Material and methods: We screened a total of 36 uterine leiomyomas from 20 patients for MED12 intron 1 and exon 2 mutations using single-strand conformation polymorphism (SSCP) method.
Results: 7 (19.44%) leiomyoma harbored a mutation in MED12 exon 2. Four fibroids (57.1%) displayed a missense mutation in codon 44 including G44R, G44S, and G44D mutations. We also observed three (42.9%) deletions including p.L36_K42del, p.V41_S52del and p.D34_ N40del. All three mutations are predicted to result in an in-frame transcript. None of the tumors displayed somatic mutations in the intron 1.
Conclusion: All mutations are predicted to be damaging at the protein level .This study confirms a major role of MED12 in the genesis of leiomyomas.

J01.28
Investigation of Diagnostic Value of Chromosome Analysis and BAC based Array CGH in Prenatal Diagnosis

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Introduction:This study aims to investigate the diagnostic value of BAC-based Array CGH and chromosome analysis in prenatal diagnosis.
Material and Methods
This study included the chromosome analysis and BAC-based Array CGH analysis of 140 amniocentesis samples with prenatal diagnosis indications.
Results
Karyotype analysis showed trisomy 21 in four patients, trisomy 18 in five patients, monosomy X in one patient, and other anomalies in three patients. The BAC-based Array CGH analysis showed four patients with trisomy 21, four patients with trisomy 18, one patient with monosomy X as numerical chromosome anomaly, and partial duplication was observed in chromosome 14 in one case as a structural anomaly.
Conclusion
The Array CGH is the most effective method to be able to complement the cases where chromosome analysis, a gold standard in prenatal diagnosis, proves to be insufficient.
Considering the inherent limitations of both methods, complementary features should be introduced in order to be able to give most accurate data at the right time

J01.29
A case of mosaic 45,X/46,XY infertile man with AZF deletion

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Introduction:Frequency of chromosomal abnormalities is approximately %15 in azoospermic men.45,X/46,XY mosaicism or mixed gonadal dysgenesis is associated with phenotypes ranging from normal male development to clinical signs of Turner syndrome.Y chromosome with structural chromosomal abnormalities is unstable and can be lost during mitosis.Therefore structural chromosomal abnormalities of Y chromosome can be observed in individuals with 45,X/46,XY mosaic karyotype.We report here a mosaic 45,X/46,XY infertile man withAZF deletion and normal male phenotype.
Methods:Karyotype analysis of peripheral blood was performed for the patient.We detected 45,X/46,XY mosaicism. FISH analysis was performed including X and Y centromere probes.Y chromosome microdeletions were investigated by polymerase chain reaction(PCR) assays.Also we investigated the AZF and SRY regions microdeletions of patient's father.
Results:Our patient was a 31-year-old male,has been married for 7 months and they were distant relatives.The patient was referred to Medical Genetics department from Urology with azospermia and infertility diagnosis.The physical examination was normal except sparse eyebrows.Karyotype analysis was 45,X[20]/46,XY[39].After applying XY centromeric FISH analysis we detected %63 XY and %37 X interphase cells.Molecular analysis revealed that AZFa and SRY regions were present but AZFb, AZFc and AZFd regions were deleted.The father's results were normal.
Conclusion:45,X/46,XY mosaic karyotype and AZF deletion were not hereditary.Genetic counselling was given to the family. We concluded that wide AZF region deletions was caused 45,X/46,XY mosaic karyotype status in our patient to reveal unstable Y chromosome during mitosis.Our aim is to emphasize that Y chromosome microdeletions should be analysed in 45,X/46,XY mosaic karyotype was detected in infertile men with normal phenotype.

J01.30
The impact of prenatal invasive genetic testing on chromosome abnormalities incidence and sex balance at birth

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One of the key roles of invasive prenatal genetic testing is to reduce the incidence of chromosomal abnormalities (ChA) among newborns. The aim of this study was to estimate the influence of invasive prenatal genetic testing on the frequency of ChA in newborns and the sex ratio at birth.
The data from central register for cytogenetic prenatal and postnatal testing were analyzed retrospectively within period 2000 - 2014. Sex ratio at birth was estimated using the data of the Statistical Office of Montenegro.
Chromosomal abnormalities were found in 100 (2.45%) out of 4072 prenatal cytogenetic analysis. Aneuploidies were the most common ChA (2.1%), and strongly correlate with the most frequent indication - advanced maternal age (61%). The most common ChA was pure trisomy 21, detected in 47 fetuses (1.1%). Unbalanced structural rearrangements were found in 13 (0.3%).
The frequency of ChA (primarily aneuploidies) among newborn infants was significantly reduced within examined period. The frequency of trisomy 21 dropped from 1 per 633 in the period 2000-2003, to 1 per 1407 newborn infants in the period 2011-2014 Constant trend of significant male predominance at birth was found within estimated period: ranging 107, 5 - 113, 4 male per 100 female newborns, and it cannot be explained as "natural appearance".
Based on a 15 years data on cytogenetic analyzes, our results contribute to the establishment of baseline data on the occurrence of ChA in newborn population. Consecutive trend of male predominance at birth may refer on possible misuse of prenatal diagnosis for the purpose of prenatal sex selection.

J01.31
Structural chromosomal abnormalities (CA) in couples with reproductive disorders: examination of the phenomenon of female predominance among carriers of reciprocal translocations

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Female predominance among carriers of reciprocal translocations (rec) has been commonly explained by male sterility, though appropriate comparative studies had not been carried out. The objectives of this study were comparative analysis of rates of balanced CA carriers among patients with infertility and among patients with recurrent miscarriages and comparative analysis of male-to-female ratio (sex ratio, SR) in the carriers. Method: Meta-analysis of data from 25 publications on prevalence of CA in couples with reproduction failures. Results: Among couples with infertility, rec carriers were found in 0.46% of males (63/13,573) and in 0.38% of females (51/13,595), SR=1.2, not different from population value of 1.06. Robertsonian translocation (rob) carriers were found in 0.52% of females and in 0.16% of males, SR=3.2, different from 1.06, $p<0.001$. Carriers of inversion (inv) were less frequent among males (0.15% vs 0.26%). Among couples with recurrent miscarriages, rec carriers were more frequent compared to those among infertile couples: 0.86% of males (113/13,192) and 1.54% of females (203/13,192), SR=0.56, $p<0.001$. Robs were found in 0.41% of males and in 0.74% of females, SR=0.55, $p<0.001$. Rate of inv was 0.14% in both males and females. Conclusion: The data obtained corroborate with the conception of female predominance among fertile carriers of rob due to male sterility. However female predominance among fertile carriers of rec can not be explained by the same reason. Firstly, there is no significant male prevalence among infertile carriers, secondly, the rate of rec carriers among infertile males is lower compared to that among fertile patients.

J01.32
Generation of divalent DNA vaccine based on p39 gene of Brucella melitensis and shiga-like toxin 2 (stx2) gene of Escherichia coli

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The virulence factors such as shiga-like toxin (Stx) and immunogenic P39 protein in Escherichia coli and Brucella melitensis, respectively are related to disease of digestive system in human worldwide. In present study the stx2 and p39 genes were cloned into expression plasmid pEEF1D-FLAG (pcDNA 3.1+) as a divalent DNA vaccine candidate. The Enterohemorrhagic E. coli ATCC 3081 and smooth virulent B. melitensis strain M5 were obtained and cultured on specific media. Bacterial DNA was extracted from colonies and used for p39 and stx2 genes amplification by PCR. The amplified products on 2% agarose gel electrophoresis revealed 285 and 1220 bp fragments for stx2 and p39 genes, respectively. Each amplified genes were T/A cloned into pGEM-T easy vector and pGEM-T-stx2 and pGEM-T-p39 were produced. The stx2 and p39 genes were sub-cloned in linearized expression vector (pcDNA 3.1+) using HindIII, XhoI and XbaI restriction enzymes and pCDNA3-stx2-p39 was generated. This final construct was confirmed by PCR and enzymes digestion. The results were showed stx2 and p39 genes were sub-cloned into pcDNA 3.1+ to generate pcDNA 3.1+-stx2-p39 recombinant vector, successfully. According to these findings novel recombinant pcDNA 3.1+-stx2-p39 construct was produced in this study could be useful as DNA vaccine candidate in animal models against shiga-like toxin producing E. coli and virulence B. melitensis strains in future studies.

J01.33
Prenatal case of 17q12 deletion detected by SNP array

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The 17q12 microdeletion syndrome is among the 10 most common microdeletions in children with unexplained neurodevelopmental delay. These microdeletions are associated with a wide range of phenotypes, including renal cyst, diabetes syndrome, genital tract abnormalities, developmental delay and learning difficulties, and more recently autism spectrum disorders and schizophrenia.
We report prenatal case of 17q12 microdeletion detected by Illumina SNP array using HumanCytoSNP-12v2.1. Generated data were analysed by Illumina KaryoStudio 1.4 and GenomeStudio V2011.1.
In the foetus with ultrasound detected unilateral multicystic dysplastic kidney de novo 17q12 microdeletion was found. A 1,4 Mb deletion of 17q12 region contained two OMIM genes ACACA (OMIM 200350) and HNF1B (also known as TCF2, OMIM 189907). The parents decided to terminate the pregnancy. The autopsy confirmed unilateral multicystic dysplastic kidney and described contralateral kidney with abnormal nephrogenesis, bicornuate uterus, vaginal atresia and mild hepatic fibrosis. The described features are consistent with Renal cysts and diabetes syndrome (RCAD, OMIM 137920). The SNP array is very useful in identifying of the small structural abnormalities of foetal chromosomes. This is important to specify prognosis of an affected foetus.

J01.34
Evaluation of genetic variations in exon4 and exon5 of RABL2B gene in infertile men with immotile short tail sperm defect.

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The immotile short tail sperm (ISTS) defect, is a syndrome which causes male infertility. Patients with ISTS disorder have immotile short-tailed sperms which can not fertilize oocyte. Numerous proteins are involved in sperm structure. One of these proteins is RAB Like 2B (RABL2B), which recently its essential role in sperm tail assembly and fertility in male mouse has been demonstrated. So its gene, which called RAB Like 2B (RABL2B), is an appropriate candidate gene in human studies. RABL2B protein has 4 GTP binding domains. Exons 4 and 5 of this gene code two of these essential domains. The purpose of this study was to evaluate the genetic variations of exons 4 and 5 of RABL2B gene in infertile men with ISTS defect and controls. In this study, 30 infertile men with ISTS defect and 30 normozospermic men as controls were recruited. Remarkably, because of the rarity of this disorder, it took 2 years to collect patients samples. To study the genetic variations, DNA was extracted from peripheral blood, then PCR sequencing was done. Sequence analysis results did not identify any mutations or single-nucleotide polymorphisms (SNPs) in exons 4 and 5 of RABL2B gene in patients and controls. Considering the fact that RABL2B is evolutionarily so conserved and exons 4 and 5 have a very important role, therefore the absence of variations is fully justified. On the other hand, as RABL2B has a high expression in testis, evaluation of other parts of this gene is recommended.
Reference:Anu sironen. TURUN YLIOPISTO. Turku 2009. Molecular Genetics of the Immotile Short Tail Sperm Defect.

J01.35
Screening of Fetal Chromosome Aneuploidies in the First and Second Trimester of 125170 Iranian Pregnant Women

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Background: Aneuploidy is one of the main causes of congenital anomalies, mental and physical problems in newborns. The aim of this study was to determine the various chromosomal aneuploidy in first and second trimester screening of pregnant women in Iran.
Methods: We conducted a descriptive retrospective study on 125170 pregnant women who were referred to a major referral medical laboratory for prenatal diagnosis (2007-2010). Patients were divided into 3groups: first trimester screening (FTS), second trimester screening (STS), and combined screening groups. In each group a questionnaire was filled out, and amniocentesis and cytogenetic analysis were carried out.
Results: Total prevalence of aneuploidy in 125170 pregnant women was one in 491, (DetectionRate=%82.7 for Down syndrome). The DR for DS in three groups were as follow: %87.5 for FTS (25783women), %80.9 for STS (91345women), and %94.7 for combined tests (8042women). Total number of cases with Edward's were 18, Patau's six, Klinefelter six, triploidy three, and Cri-du-chat syndrome one cases.
Conclusion: The present study shows the frequency of aneuploidy in first and second trimester screenings in a major medical laboratory in Tehran. The prevalence of aneuploidies grows with increased maternal age. The rate of aneuploidy in first trimester is higher than second.

J01.36
Genetic variants of KITLG, SPRY4 and BAK1 genes determining increased risk of testicular germ cell tumors in fertile man and patients with infertility

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Introduction: More recently, genome-wide association studies (GWAS) have reported association of testicular germ cell tumors (TGCTs) with KITLG, SPRY4, BAK1 genes.
Materials and Methods: The aim of this study was to determine alleles and genotypes of high risk for patients with infertility for the identification of the TGCT risk group for clinical monitoring. Five SNPs mapping to SPRY4 (rs4624820, rs6897876), KITLG (rs995030, rs1508595) and BAK1 (rs210138) were genotyped in 77 fertile men and 71 unfertile patients with AZF deletion on the Y chromosome.

genes	Allele	Risk allele	Controls	Cases	Per allele OR (95% CI)	Heterozygote	Homozygote	p trend
SPRY4								
rs4624820	A/G	A	24/38/15	19/38/14	0.91(0.58-1.44)	1.07(0.46-2.52)	0.85(0.33-2.18)	0.68
rs6897876	C/T	C	28/33/16	21/32/18	0.79(0.50 -1.26)	0.86(0.38-1.98)	0.67(0.28-1.61)	0.35
KITLG								
rs995030	G/A	G	45/30/0	46/25/0	1.17(0.65-2.11)	0.82(0.42-1.60)	1.23(0.63-2.40)	0.55
rs1508595	G/A	G	43/29/4	35/30/6	1.31(0.78-2.19)	1.45(0.37 -5.67)	1.84(0.48 -7.05)	0.31
BAK1								
rs210138	G/A	G	0/24/53	0/30/41	1.45(0.80-2.63)	1.45(0.80-2.63)	1.29(0.03-6.35)	0.16

Results: The combination of high risk genotypes for all three genes (ACGGG) was detected in 4/77 normal fertile men and 10/71 patients with infertility and AZF deletion. We were unable to identify the significant increase in the frequency of high risk genotypes combinations among the patients with infertility compared to normal fertile men.
Conclusions: We identified infertile patients with a combination of high risk genotypes for all genes, KITLG, SPRY4 and BAK1. These patients may have an increased risk of developing TGCTs, which should be considered for further consultation and monitoring. Genotyping of patients in clinical high-risk groups (infertility, cryptorchidism and microlitiasis) might be an additional factor for an individual prognosis.

J01.37
Mutation in SYCP3 gene in a woman with recurrent miscarriage

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Introduction
Aneuploidy, a chromosomal numerical abnormality in the conceptus or fetus, occurs in at least 5% of all pregnancies and is the leading cause of early pregnancy loss in humans.
SYCP3 gene encodes an essential structural component of the synaptonemal complex. This complex is involved in synapsis, recombination and segregation of meiotic chromosomes.
Mutation of the SYCP3 gene have been shown to generate an aberrant synaptonemal complex and might be lead to recurrent pregnancy loss.
Material and Methods
We report a 33 years old woman with 2 miscarriages with aneuploid fetus. All known causes, such as hormonal, structural, immunological and coagulation disorders were excluded. Karyotypes (patient and husband) were normal.
The SYCP3 657T>C mutation was examined using PCR performed under standard condition.
Results
Mutation 657T>C was identified in SYCP3 gene.
Conclusion
Mutation in SCYP3 gene may be associated with recurrent miscarriage caused by fetal aneuploidy. Further studies are needed in women with recurrent pregnancy loss to confirm our conclusion.

J01.38
MTHFR gene A222V mutation as marker of risk of pathology of the embryonal development

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Genotyping of a mutation of A222V (677C/T) of a MTHFR gene which according to the available data is an objective marker of the complicated course of pregnancy and violation of an embryonal development is carried out. For research samples of genomic DNA of 127 women (from 18 to 50 years) the residents of Novosibirsk selected by the epidemiological principles and 102 women (from 20 to 44 years) with the stood pregnancy or not incubation of pregnancy are used.
In control group of women there were tipirovana all three possible a genotype of the analyzed MTHFR gene locus: CC (53,54%), CT (39,38%) and TT (7,08%) which distribution corresponded to Hardy-Weinberg expected at balance. Frequency allele 677C made 0,732, and allele 677T - 0,268. In group of women at whom pregnancy ended with an abortion or the stood pregnancy, there were genotipirovana three possible genotypes which frequencies however had reliable differences from the first group of the surveyed: CC (37,25%), CT (47,06%) and TT (15,69%) with a frequency allele 677C - 0,608, and allele 677T - 0,392. The comparative analysis of the surveyed groups showed higher frequency of prevalence of a mutation of A222V (as in a heterozygotic, and homozygous form) among women with reproduction pathology. On the one hand it confirms the predictive importance of this genetic marker (especially MTHFR TT genotype), and on the other hand testifies to an imperative need to pay the closest attention to a hypergomocysteinemiya problem among women with pathology of a reproduction and pregnant women.

J01.39
PLACENTAL GROWTH FACTOR AS A PROGNOSTIC MARKER FOR PREECLAMPSIA AND GENETIC VARIANTS LEVEL OF PIGF IN PREGNANT WOMEN WITH PREECLAMPSIA

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Placental growth factor (PlGF) is one of the most important regulators of the formation of the placenta and its vascularization villi.
The study included 70 pregnant women with preeclampsia mild to severe and 50 healthy pregnant women for all patients was carried out molecular genetic testing of susceptibility genes to preeclampsia. Low levels of PlGF (below 100 pg / ml) in the serum of pregnant women testified about the high risk of developing preeclampsia, which is confirmed by correlation between the levels of PlGF and the severity of preeclampsia (r = 0,43). The association of molecular genetic markers of genes folate cycle, clotting factors, vascular endothelium, vascular gene to the level of placental growth factor in pregnant women with preeclampsia. Found that pregnant women with the presence of the mutant allele of the MTHFR gene 677T genotype and AGT 521T, as well as having homozygous (TT) eNOS3 gene, marked the lowest concentration of PIGF 76,3 pg / ml and 87.3 pg / ml. and 43.2 pg / ml., respectively. The proportion of placental growth factor was the highest in women with mutations in the genes: F5 1691A (254,3 pg / ml) and F2 20210A (217,6 pg / ml). Also in the group of pregnant women with preeclampsia with genotypes - MTHFR A1298S and AGT T704S, the level of PIGF remains within the norm (128.7 pg / ml and 143.5 pg / mL, respectively).

J01.40
The role of miRNA polymorphisms in recurrent pregnancy loss: a case control study

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Recurrent pregnancy loss (RPL) has been traditionally defined as two or more clinical aberrations pregnancies lost before the 20th week of pregnancy in humans that occurs in 1 in 100 pregnancies around the world. Several data suggest that miRNAs may be essential for the normal function of the reproductive system. Polymorphisms in the pre-miRNAs may have negative effects on mature miRNA processing and expression. The purpose of the present study was to investigate the association of miR-196a2T>C (rs11614913) and miR-499A>G (rs3746444) polymorphisms with RPL in Iranian women. We used PCR-RFLP and Tetra ARMS PCR for genotyping of miR 499aT>C and miR 196a2C>T in 83 women with RPL and 100 fertile women with no history of miscarriages respectively. There was no association between miR-499A>G polymorphism and RPL (OR=1.43, 95% CI=0.36-5.67; P=0.61). However, significant difference in distribution of miR-196a2 rs11614913 ge¬notypes was found in RPL patients in comparison to controls (OR=2.69, 95% CI=1.03-7.03; P=0.04). The present study shows an association between miR 196a2C>T polymorphism and RPL in Iranian women with RPL.

J01.41
The study of AZFa microdeletions in Iranian patients with non-obstructive azoospermia

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Infertility is defined as the inability to fertile after 12 months of sexual intercourse without using any contraceptive method. Y-chromosome microdeletions are among the most important genetic factors causing male infertility with different frequencies in azoospermic men. According to the European Academy of Andrology (EAA) and the European Molecular Genetics Quality Network (EMQN) guidelines molecular analysis of the AZFa region involves the use of the two STS markers sY84 and sY86. In the present study 50 azoosperm patients who had no deletions in the AZFb and AZFc region were studied for deletions in the AZFa region. 50 men who had at least one child were considered as the control group. Microdeletion frequencies were calculated using the Multiplex PCR for SY81, SY746, SY742, USP9Y, DDX3Y, SY86, SY84 STS markers. The results indicated the deletion of USP9Y, SY81, DDX3Y markers with the frequency of 4% for each one. However, no deletion was observed for sY84 and sY86 markers. There was no deletion in the control group. Based on the present results, it seems that the type of Y chromosome microdeletion in the AZFa region may be different based on racial differences in different populations. Therefore, the effective AZFa markers for the diagnosis of male infertility may be different at least in Iranian infertile men.

J01.42
EFFICIENCY OF PRENATAL DIAGNOSIS OF DUCHENNE MUSCULAR DYSTROPHY AND SPINAL MUSCULAR ATROPHY FOR LAST 5 YEARS IN MOLDOVA

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Introduction. The monitoring and prevention of hereditary diseases are the current tasks of medical genetics and health. Prenatal diagnosis (PD) is one of the methods proposed. Muscular dystrophy Duchenne/Becker (DMD/B) and spinal muscular atrophy (SMA) are neuromuscular hereditary disorders that are frequently encountered in Moldova and can be detected through prenatal diagnosis (PD).
Methods. After medico-genetic consultation pregnant women with high risk of hereditary disease DMD/B and SMA undergo PD, which Moldova is achie-

ved by methods such as amniocentesis and then multiplex PCR, PCR/RFLP, the primer sets specific and polymorphic sites.
Results. We present a retrospective study for five years of PD of neuromuscular disorders with high frequency in Moldova. In total were performed 15 prenatal diagnoses: 9 - DMD/B and 6- SMA during 2010-2014. Based on the results the indirect diagnosis is more useful in Moldova. Thus were detected 4 affected fetuses, 3 of them were with DMD/B and 1 with SMA. Have been analyzed the strategies for prenatal diagnosis of these diseases in different countries.
Conclusion. Prenatal diagnosis is an effective way to prevent the birth of children with hereditary diseases. In the last 5 years thanks to efforts to raise public awareness is notice an increase of the number of appeals for prenatal diagnosis The methods of modern (MLPA etc.)molecular genetics of PD raise the efficiency and precision to 98%. Efficiency of prenatal diagnosis in Moldova is 71,4%. Analyzing different diagnostic methods used in the world was proposed the method which will be better implemented in Moldova.

J01.43
DETERMINATION OF HUMAN SPERM DNA FRAGMENTATION WITH AN IMPROVED SPERM CHROMATIN DISPERSION (SCD)TEST

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Objectives: To improve SCD protocol and develop it as a simple kit for the determination of sperm DNA fragmentation.
Study subjects: 50 human semen samples with concentration more than 5 million per ml, at the Department of Biomedical - Genetics, Hanoi Medical University.
Methods: Using sperm chromatin dispersion technique to assess human sperm DNA fragmentation as protocol discribed by Fernandez (2003).
Results: We determined the range of chemical concentrations in the lysis solution and the sperm DNA fragmentation of 50 human semen sample by using the improved SCD kit, and the sensitivity and specificity of the improved SCD (>95%).
Conclusion: We improved suscessfully a SCD protocol in the lysis solution components and developed it as a simple kit with specificity and sensitivity comparable to Halosperm commercial kit for assessing human sperm DNA fragmentation.

J01.44
Aloe vera-induced modulation of nitric oxide signaling pathway and androgenesis in the testis rat leydig cells

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Background: Inducible nitric oxide synthase (iNOS) through its product, nitric oxide (NO), contributes to the induction of germ cell apoptosis. This study was designed to investigat the effects of Aloe vera gel on male rat reproductive organs and its relation with serum NO and expression of iNOS gene in testis cells.
Methods: 36 adult male Wistar rats were designed into 3 equal groups. The rats received normal saline (control) or Aloe vera gel at doses of 150 and 300 mg/kg, orally for 60 days. Then they were mated with untreated female rats. Then reproductive organs of rat weights, semen quality, serum testosterone concentrations in male rats and the rate of fertility in female rats were assessed. At the same time, the in vivo capacity to produced NO, was measured by Griess method and gene expression for iNOS was also studied by Real Time-PCR.
Results: A PCR product of the expected size amplified iNOS mRNA gene was expressed in testis cells and increased the serum NO level after 60 days in the treatment groups, in compared to control. Furthermore, the weights of the testes, seminal vesicle and the sperm count of male rats and serum testosterone concentrations as well as the pregnancy rate were reduced in experimental groups compared to control.
Conclusion: These results may suggest that AVG has adverse effects on the reproductive system of adult male rat through NO signaling pathway. Therefore, it is recommend to be avoided in male subjects in whom the reduction in reproductive capacity is important.
Lue Y, et al,. J. Endocrinol. 2003; 144: 3092-3100

J01.45
The importance of MTHFR gene mutation test in patient with recurrent miscarriages and normal homocystein level

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Homocysteine is an enzyme encoded by MTHFR (methylenetetrahydrofolate reductase) gene located on chromosome 1. Mutations in MTHFR gene may result in the afflicted metabolism of homocysteine and thus might increase the risk of recurrent miscarriages. In some cases, recurrent pregnancy loss could be prevented by prescribing folic acid and B group vitamin supplements. The demand of MTHFR gene sequencing for variations is commonly overlooked by doctors or genetic counsellors. To highlight this problem we present a case study of recurrent miscarriages in a patient with a homozygous c. 655C>T variation in MTHFR gene with clinically normal homocysteine level (11.2 mmol/l). There is the need of molecular genetic testing for MTHFR gene variations in patients with recurrent miscarriages because treatment of hyperhomocysteinemia exist.

J01.46
Prenatal and postnatal correlations in velo-cardio-facial syndrome

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DiGeorge syndrome (velo-cardio-facial) is the most common microdeletion syndrome(22q11.2) in humans, having the incidence about 1/4000 newborns. It is diagnosed in about 1/68 cases of heart malformations(Wilson et al.,1994). It is characterised by a broad phenotypic variability, having important variability even among the same family affected members and between identical tweens.Frequent could be associated heart,kidney malformations, palatal anomalies, immune deficits. Comportamental problems and mental retardation are resent in about 46% of cases, with different severity , including delay of motor and verbal aquisition, autistic features , psichiatric disorers as depresion, bipolar affection and schisofrenia.
In our prenatal cases diagnostic suspicion was ultrasound diagnosis of heart malformations as truncus arteriosus and tetralogy of Fallot. In a newborn case, medical history of polyhydramnios, typical facies and heart malformation, in this case interrupted Aortic arch, rised suspicion of this syndrome. All cases were confirmed using arrayCGH and FISH technique.
In conclusion, presence of heart malformations in prenatal diagnosis it"s an important marker of a possible genetic syndrome. Additional, using of microarray analysis would allow as an increased number of microdeletion or microduplication syndromes to be diagnosed. Apart from prognosis of the heart malformation, clinical variability and variable association of intellectual disability, represents a challenging step in prenatal counselling of families.

J01.47
Prenatally Diagnosed Double Aneuploidy: 48, XYY+21

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The incidence of trisomy 21 is significantly correlated with maternal age and the presence of an extra Y chromosome in males is common occurrence,however the co-existence of both aneuploidies in patient is a rare phenomenon.We report prenatal diagnosis of 48,XXY,+21 karyotype on a fteus demonstrating increased nuchal translucensy measurement with ultrasoud scanning using amniocyte culture.The double aneuploidy was subsequently confirmed by FISH and QF-PCR.An amniotic fluid sample prepared from a 41year old woman with Azeri Turk ethnicity was received for cytogenetic analysis.The amniocyte culture and chromosomal analysis was performed using standard cytogenetic techniques.Uncultured amniocyte cells prepared from patients amniotic fluid sample on microscope slides by dual and three colore interphase FISH. Confirmation of the detected aneuploidy carried out by QF-PCR.Chromosome analysis of cultured amniocytes demonstrated 48,XYY,+21 in all G-banded cells. Analysis of hybridized cells with specific probes for the 5 clinicaly improtant chromosomes showed a single green signal for chromosme X,two red signal for chromosome Y.On the other hybridisation area three red signals specific for chromosomes 21.QF-PCR results confirmed 48,XYY+21, with extra 21 originating of second meiotic failure.Aneuploidies are due to non-disjunction at meiosis or post zygotic mitosis.The XYY occurs when sperms containing 2 copies of chromosome Y fertilize an ovum containing 2 copies of chromsome 21 instead of one. Non-disjunction leading to formation of 24,YY occure at paternal meiosis II

or mitosis, where Non-disjunction during maternal meiosis I is the common cause of trisomy 21.Complete disomy of chromosome Y and full trisomy of chromosome 21 detected in patient appears to be the result of meiotic non-disjunction occured during gametogenesis

J01.48
Comparison of orthodontic disorders is mono- and dizygotic twins

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Introduction:
Twin studies are one of the most effective methods of evaluating the interaction of genetic and environmental influences on a particular phenotype also in orthodontics. This study aims to compare the differences in dental and skeletal morphology in mono- and dizygotic twins.
Materials and methods:
10 sets of monozygotic (5 girls and 5 boys, mean age: 18.5 years) and 10 sets of dizygotic twins (same gender, 3 girls and 7 boys, mean age: 19.7 years) were assessed. Cast analysis, orthopantomographic and lateral cephalometric radiographs were evaluated for each subject.
Results:
Dimension and shape of the upper anterior teeth were the same in 99.69% of the monozygotic and 93.91% of the dizygotic twins. Monozygotic twins presented differences in the upper transversal width both in the molar and canine region. Whereas, dizygotic sets had different statical occlusions in the transversal and saggital planes, too. In 50% of the monozygotic and dizygotic sets, symmetrical dental position, while in 50% "mirror image" could be observed. Cephalometric analysis revealed slight differences in interincisal and lower central incisor inclination in both mono- and dizygotic sets.
Conclusions:
The results of this study explain the genetically inherited patterns of dental and skeletal disorders.

J01.49
Preimplantation Genetic Diagnosis (PGD) for monogenic disorders and chromosome rearrangements: an innovative approach for increased diagnostic efficiency and patient benefit.

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Introduction: PGD is traditionally performed by biopsy of cleavage-stage (Day 3) embryos, genetic testing of the biopsied material, and fresh embryo transfer. This approach provides limited time for analysis and interpretation of genetic testing, and for discussion of results with the couple; clinically there is also a risk of ovarian hyperstimulation syndrome (OHSS) and high multiple pregnancy rate.
Materials and Methods: 161 couples who were carriers of either monogenic diseases (n=127) or chromosomal rearrangements (n=34) underwent oocyte retrieval after controlled ovarian stimulation using a protocol optimised for reduction of OHSS. Trophectoderm biopsy of day 5 or 6 blastocysts was followed by vitrification. DNA analysis of biopsies was by whole genome amplification and Preimplantation Genetic Haplotyping for monogenic diseases or array CGH for chromosome rearrangements. Following diagnosis and patient counselling, single embryos were warmed and transferred.
Results: 85 pregnancies were achieved (53% per couple) with no cases of mild or severe OHSS (0%), one monozygotic multiple pregnancy (1.1% per pregnancy, similar to the natural incidence of multiple pregnancy) and high patient satisfaction. These results compare favourably with those in the prior 12 months, when 11 patients (4%) developed OHSS (P<0.001) and the multiple pregnancy rate was 13% (P<0.001). Testing throughput has increased from five to nine couples per week, delivering an 80% increase in productivity with no increase in staff numbers.
Conclusion: This novel strategy delivers efficiencies and patient benefit; embryo warming and transfer can be scheduled to allow time for robust diagnosis and patient results counselling.

J01.50
Large nuclear vacuoles as a possible marker of sperm DNA fragmentation

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ICSI as a part of assisted reproductive technologies allows infertile men to father a child bypassing natural selection barriers. Sperm selection mainly relies on its morphological characteristics. Sure, the spermatozoa with normal morphology or with light pathology would be selected for fertilization. But in case of severe teratozoospermia one should choose the most appropriate sperm from those with abnormal morphology. So, to know if the sperm morphology reflects its DNA fragmentation was the aim of this study since the sperm DNA fragmentation is associated with reduced embryo quality, implantation and live birth rates.
Sperm samples from two men with normal somatic karyotypes and teratozoospermia were collected by masturbation. Shortly after ejaculate liquefaction under the high magnification the spermatozoa with large nuclear vacuoles were selected. As a control the spermatozoa with normal morphology were used. To analyze sperm DNA fragmentation TUNEL assay was used. Among sperm with large nuclear vacuoles DNA fragmentation rate was extremely higher compare to the control - 14,29% vs. 1,12% (p = 0,0008).
So, large nuclear vacuoles in sperm head may be a morphological marker of its DNA fragmentation. Understanding such a relationships helps to select spermatozoa with intact genome to improve fertilization efficiency and embryo quality.

J01.51
Prenatal Diagnosis of Partial Monosomy 13q and Partial Trisomy 10q: A Case Report

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We report a prenatal case the combination of partial monosomy 13q and partial trisomy 10q which were detected by oligonucleotide array comparative genomic hybridization (aCGH). Chorionic villus sampling was done on 34-year-old woman at 13 weeks of gestation due to abnormal maternal serum screening and increase in nuchal translucency. The parents had a healthy daughter and there was no family history of miscarriage and neonatal deaths. The fetal karyotyping on chorion villus showed normal female karyotype 46,XX. The DNA was extracted and directed to Biobank. It was decided to continue the pregnancy. However, ultrasound examination at 21 weeks of gestation showed holoprosencephaly, microcephaly, soft tissue swelling of the head and trunk of the fetus. By means of oligonucleotide aCGH analysis (SurePrint G3 Human CGH Microarray Kit, 4x180K, Agilent) we identified a 15 Mbp duplication 10q26.11q26.3(120678170-135404523) and a 22 Mbp deletion 13q31.3q34(93390362-115059020) which were previously described as associated with these malformations in the fetus. After genetic counseling parents decided to terminate the pregnancy. Cytogenetic analysis revealed that the father was a carrier of the balanced translocation 46, XY,t (10;13)(q26.1;q31.3). Actually, aCGH analyses may be useful for detected unbalanced karyotype in fetuses with increased risk for chromosomal abnormalities at prenatal genetic counseling. In our case, this led to the identification of an unexpected parental translocation.

J01.52
Cytogenetic study of miscarriages after IVF and natural conception in women aged under and over 35 years

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We compared the frequency and the spectrum of karyotype abnormality in the first trimester miscarriages in women, who conceived naturally (NC) and who conceived through in vitro fertilization (IVF). Patients were subcategorized by their age: <35 years (NC, n=173; IVF, n=108) and ≥35 years (NC, n=107; IVF, n=111). A total of 499 miscarriage karyotypes was analyzed. The spectrum and the relative proportions of different cytogenetic categories in karyotypically abnormal miscarriages differed neither between the NC and IVF patients aged <35 years, nor between the NC and IVF patients aged ≥35 years. In the patients aged <35 years, the incidence of abnormal miscarriage karyotype was lower in the IVF group (37.04% vs 62.43%). In the patients aged ≥35 years, the incidence of miscarriages with cytogenetic pathology did not differ between the NC and the IVF group (75.70% vs 58.56%). The lowest frequency of karyotypically abnormal miscarriages (29.82%) was detected in the young IVF-treated patients at <7 weeks of gestation.
Thus, IVF does not increase the risk of a pregnancy loss because of abnormal embryonic karyotype, nor does it increase the preponderance for any specific type of cytogenetic abnormality in both patients aged under and over 35 years. In young IVF-treated women early pregnancy loss is generally

caused by non-cytogenetic factors. Identification of a cytogenetically normal spontaneous abortion is clinically significant and reinforces the importance of developing an appropriate diagnosis and treatment strategies for IVF patients in order to reduce the risk of euploid pregnancy loss.
Supported by RFBR and scholarship from RF President.

J01.53
Ambiguous findings in prenatal aneuploidy screening by commonly used QF-PCR assays: a 2 year long Czech prospective study and need for compilation of marker abnormal patterns.

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The Devyser Resolution v.2 QF-PCR assay (Devyser, Sweden) occasionally detects submicroscopic duplications which may suggest a "trisomic pattern" for a single marker, while the remainder indicates a normal chromosome complement. The CMGS/ACC Best Practice Guidelines (v2.01) state that abnormal marker patterns, flanked by normal results, may reflect presence of CNVs. We carried out a prospective study on amniotic fluids referred for QF-PCR testing for common aneuploidies, in parallel with karyotyping. Of the 271 prenatal samples received from 1/2014 to 1/2015, 6.3 % were aneuploid by QF-PCR testing and validated by karyotyping. In 9 female fetuses (46,XX) ZFY was found, which is normally present on the Y chromosome. This phenomenon will be further examined upon receiving parental DNA. In addition, we revealed a signal for marker "D18S386" located at locus 18q22.1. Parental DNA analysis was normal in all tested cases and proved that the extra peak in "D18S386" represents the missing paternal allele of marker "D13S305" (13q13.3). Aforementioned, markers have not been previously reported as representing inherited CNVs identified by markers from the QF-PCR assay under study. In this regard it is important to develop databases compiling such CNVs in order to assess their pathogenic impact in commonly used QF-PCR assays, and this information could reduce the need for follow up resampling of parents. Finally, trisomic markers not flanked by "normal" markers could indeed represent a clinically significant duplication which needs to be followed and reported to respective international databases. Supported by: FNM00064203, CZ.2.16/3.1.00/24022, NF-CZ11-PDP-3-003-2014, IGA NT13770.

J01.54
Morphological markers of sperm DNA fragmentation

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Semen analysis is widely used to assess male fertility but it tells nothing about sperm DNA quality. Spermatozoa with fragmented DNA as believed are able to efficiently fertilize an egg reducing embryo quality and implantation rates. Searching some morphological markers of sperm DNA fragmentation allows IMSI to be recommended to exclude gametes with damaged DNA from fertilization.
As for this we analyzed sperm DNA fragmentation rate among patients with different predominant sperm head shapes.
Semen samples were collected from 80 subfertile males. Sperm head morphology was evaluated according to Kruger's strict criteria. The following head forms were identified: big, small, round, bulb, amorphous, elongated, double, spot, with abnormal acrosome, vacuolated, with a light pathology, normal. Sperm DNA fragmentation rate were analysed TUNEL assay. 2000 spermatozoa per sample were evaluated.
The highest sperm DNA fragmentation rate was observed for those who had spermatozoa with large nuclear vacuoles as a predominant sperm head shape (p≤0,0001). And the correlation was established between these two parameters (r=0,33, p≤0,05).
So large nuclear vacuoles may be a possible marker of sperm DNA damage and for those patients who had such shape as a predominant IMSI should be recommended.

J01.55
The role of single nucleotide polymorphisms in developmental genes in the etiology of anorectal malformations

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Introduction: Anorectal malformations (ARM) are one of the most frequently occurring birth defects of the gastrointestinal tract. In the majority of patients, multiple congenital malformations are present. The etiology remains unknown, but is likely to be heterogeneous with both environmental and genetic factors playing a role. The aim of this study was to test the association of ARM with two known polymorphisms in the developmental genes *BMP4* and *GLI2* in a large group of Caucasian patients, as well as the gene-gene interaction.
Material and methods: A case-control study was performed in a Caucasian population of 431 nonsyndromic ARM patients and 661 population-based controls. Patients and controls were derived from the AGORA (Aetiologic research on Genetic and Occupational/environmental Risk factors for Anomalies in children) data- and biobank of the Radboudumc Nijmegen, The Netherlands. Genotyping was performed using Kompetitive Alleles Specific PCR assays.
Results: The non-synonymous SNP rs17563 in *BMP4* was not associated with ARM, but we did find associations between ARM and the non-synonymous SNP rs3738880 in *GLI2*, especially in ARM patients with multiple congenital malformations (homozygous GG genotype: OR=2.1; 95%CI: 1.2-3.7). A 4-fold increased risk was shown when homozygous variant genotypes of both genes were present (OR=4.1; 95%CI: 1.0-17.8).
Conclusions: We showed independent associations between the variant in *GLI2* and ARM in patients with multiple congenital malformations. In addition, a gene-gene interaction was demonstrated between *BMP4* and *GLI2*, both downstream genes in the Sonic Hedgehog (SHH) pathway, which is involved in several important developmental processes.

J01.56
Interaction between *MTHFR* 677C>T and periconceptional folic acid supplementation in the risk of hypospadias

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Background
Hypospadias is a common congenital malformation of the penis with a dislocation of the urethral opening. It has a multifactorial etiology. Folate is important during embryogenesis. Folate levels are reduced by the C677T polymorphism in the methylenetetrahydrofolate reductase (*MTHFR*) gene, which is involved in the etiology of several birth defects, but was never studied for hypospadias. Maternal periconceptional use of folic acid supplements may compensate for reduced folate levels and play a role in the prevention of birth defects. Although this was confirmed for some congenital malformations, results for hypospadias are inconsistent. Therefore, we investigated independent associations and interactions of maternal folic acid use and maternal and infant *MTHFR* C677T polymorphisms with risk of hypospadias.
Methods
We conducted a case-control study among 697 nonsyndromic hypospadias cases and 711 controls from the AGORA data- and biobank. Information about folic acid use was derived from maternal questionnaires and DNA samples from mother and child were used to genotype the *MTHFR* C677T polymorphism. In the analyses, we assumed a dominant effect of the polymorphism.
Results
Preliminary univariable analysis showed a small protective effect of maternal periconceptional use of folic acid supplements on hypospadias risk (odds ratio (OR)=0.8, p=0.05). No associations were found for the infant or maternal *MTHFR* C677T polymorphism. However, lack of folic acid supplement use in combination with carrying the *MTHFR* C677T polymorphism increased hypospadias risk (infant or maternal polymorphism: OR=1.6, p=0.01).
Conclusion
This study showed an increased risk of hypospadias when no folic acid supplements were used and mother or child carried the *MTHFR* C677T polymorphism.

J01.57
Prenatal SNP-array trio analysis; overview of the past two years

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Danish pregnant women are offered a risk assessment at gestational week 11. The risk assessment is based on ultrasound, blood sample and an individual clinical assessment.Danish fetal obstetricians have in collaboration with Danish Society of Medical Genetics agreed on three general criteria for at risk pregnancies (nuchal translucency> 3.4, any malformation, and small for gestational age). The past two years we have offered prenatal SNP-array analysis to pregnancies with increased risk. During this period our department was the only in Denmark offering prenatal trio SNP-array analysis.
This analysis is performed as a trio analysis where we analyze CVS or AF, and both parents at the same time. The result from the analysis was reported within a week.
Results:
In 2013 we analyzed 40 samples and in 2014 89 samples. Of these 129 samples, 21 abnormal samples were detected, of which 10 would not have been detected by traditional karyotyping.
Conclusion:
SNP-array is a fast and excellent method for prenatal analysis.
The advantages of SNP array trio analysis are quick results, exclusion of maternal contamination, detection of uniparental disomi, and correct identification of the sample.
Our results describe that SNP-array detects 8% more clinically significant results than traditional karyotyping.

J01.58
CHROMOSOME ABERRATIONS IN FETAL SAMPLES: AN INDIAN SCENARIO

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Chromosome analysis is carried out in fetus for prenatal diagnosis and in recurrent miscarriages. Genomic analysis by employing conventional G-banding enables numerical and structural analysis on all chromosomes. FISH is considered as an adjunct for rapid detection of aneuploidies and also in a situation of unsuccessful outcome of tissue culture. In the present study, G-banding and FISH analysis were carried out on equal number of prenatal and abortus samples. The result was categorized in three age groups (maternal) as presented in the table below. FISH-detected abnormalities were significantly lower in both prenatal and abortus samples, most likely due to its limitation on structural changes. Fetuses of mothers below 25 years showed maximum aberrations followed by the elderly mothers above 35 years of age. Early marriage and multiple conceptions at early age could be the underlying factor. In abortuses, similar trend was observed though frequency was high in all age groups. G-banding analysis has detected significantly higher number of aberrations, which has been favored by detection of structural rearrangements. The report strongly advocates employment of conventional analysis in prenatal diagnosis and also recurrent miscarriages.

Table 1

Method/ Sample	Result	Age groups (years)						Total [%]
Prenatal		<25		25-35		>35		
G-band		M	F	M	F	M	F	
	N	3	6	49	28	34	26	146
	Ab	1	2	0	1	8	5	17 [10.4]
	Abn	XX/XY [1]	Inv(9) [1]; der(9)t(9p13;21q22)mat, -21 [1]	0	DS [1]	DS [3]; PS [1]; der(22)t(9p13;22q13)mat [1]; del(5p15.2) [1]; t(14;21),t(13;18) [1]; t(1;3)(p32;q27) [1]	DS [2]; PS [1]; inv(9) [1]; del(9q13) [1]	10 types
FISH	N	6		45		42		93
	Ab	0		1		1		2 [2.1]
	Abn	0		+13		+21		2 types
Abortus								
G-band	N	2		20		3		25
	Ab	2		28		1		31 [55.3]
	Abn	XX/XY [1]; del(Xq) [1]		TS [5]; PS [3]; ES [3]; DS [10]; +11 [1]; t(9;13) [1]; triploidy [5]		DS [1]		9 types
FISH	N	14		113		15		142
	Ab	10		43		7		60 [32.9]
		PS [3]; ES [1]; DS [2]; TS [2]; KS [1]; XX/XY [1]		PS [14]; ES [6]; DS [13]; TS [6]; triploidy [4];		PS [1]; ES [1]; DS [5]		7 types
Total	N	31		255		120		406
	Ab [%]	15 [32.6%]		73 [22.2%]		22 [15.5%]		110 [21.3]

M Male; F Female; N Normal; Ab Abnormal cases; Abn Aberrations; PS Patau syndrome; ES Edward syndrome; DS Down syndrome; TS Turner syndrome; KS Klinefelter syndrome; FISH without sex chromosomes

J01.59
Genomic imbalances leading to disruption in spermatogenesis in patients with idiopathic male infertility

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Introduction: The infertility affects 10-15% of all couples. In about 40% of the cases the male factor is reported to be the main reason for unsuccessful fertilization and 30% of the male infertility is idiopathic. In the current investigation we report microarray analysis data of the infertile males with azoospermia and oligoasthenoteratozoospermia.
Materials and methods: We tested 24 patients with idiopathic infertility by array CGH.
Results: We found deletions in 4 autosomal loci. The deleted region in 8p23.1 includes SPAG11B and SPAG11A-genes, associated with spermatozoa maturation and fertilization.
Deletion in 14q11.2 (EDDM3A and EDDM3B) could affect the synthesis and secretion of epididymides-specific proteins in nonobstructive azoospermic men.
The loss in 17q21.31, observed in two patients, encompasses TTLL6 - gene, important for sexual differentiation, spermatogenesis, and male fertility.
The deletion in 8p11.22, detected in two patients, affects two genes ADAM5 and ADAM32. The ADAM32 gene is expressed predominantly in the testis, suggesting a potential role for ADAM32 in sperm development or fertilization.
Conclusion: By aCGH are revealed specific microstructural aberrations in 8th and 14th chromosomes, with possible association with infertility. We suggest 7 potential candidate genes with presumed important biological significance in spermatogenesis, sperm capacitation and fertilization. This could contribute to more accurate diagnosis of infertility and personalized approach to infertile males.
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J02.01
Study of demographic features and VSX1 mutations(1 and 5 exons) in patients with keratoconus in Chaharmahal va Bakhtiari province using PCR-SSCP and DNA Sequencing

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Objective: Keratoconus (KC) is a disorder in which the cornea is swollen and thined and deformed. Many genes involved in the disease, but as evidence of the role of genes in the etiology of KC VSX1 is more sicnificant. Also in

some studies mutation in this gene have not been associated with disease. This study aimed to survey demographic features and determine the frequency of mutations in exons 1 and 5 of VSX1 in Chaharmahal va Bakhtiari province.
Materials and Methods: In this study mutations in two exons mutations 1 and 5 genes of VSX1 in 100 patients with keratoconus reviewed. DNA was extracted using standard phenol-chloroform method and PCR-SSCP screening for mutations in these genes had been confirmed. Also demographic features such as sex,age and place,…had been colleted by Questionnaire and analyzed by SPSS.
Results:In this study the prevalence of KC was about similar in men and women.(51% and 49%).many of them lived in urban area(75%). In the case of patients were no mutation in exon1 but we found a novel polymorphisms in 9 patients with c.1001A>G in exon 5.
Conclusion: Our investigation showed that the gene mutations associated with keratoconus disease, VSX1 mutation in very small samples of the disease in patients with keratoconus in Chaharmahal va Bakhtiari and clinical importance in this area is unconsiderable. also we found that changes in this gene are vary to different areas. Further investigation of these genes and his relationship with keratoconus will determine the necessary information for the prevention and management of these genes provides visual disturbances

J02.02
Association between MIF gene variation and response to glucocorticoid treatment in sudden sensorineural hearing loss

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Subject: Several lines of evidence suggest the role of immune system in pathogenesis of sudden sensorineural hearing loss (SSNHL). Macrophage migration inhibitory factor (MIF) mediates its role in various immune and inflammatory conditions by regulation of immune reactions. Several studies have confirmed an association between MIF gene polymorphisms and susceptibility to various inflammatory and autoimmune disorders. The aim of this study was to explore the association between MIF (-173 G/C) polymorphism (rs755622) and SSNHL in an Iranian population.
Methods: In this case-control association study, SSNHL cases (N=77) were included. Normal healthy subjects (N=100) were also recruited from the same region. Genotyping for MIF (-173 G/C) polymorphism was carried out using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique.
Results: The association between MIF gene polymorphism in relation to hearing recovery after treatment with GC was investigated in patients with no improvement (N=38) in hearing loss compared with the group showing response to GC treatment (N=39). The frequency of the MIF -173 C allele carriers (GC+CC genotype) was significantly elevated in SSNHL patients who responded to glucocorticoid treatment compared to the patients with no response to treatment. (GC+CC vs GG) (p=0.02, OR=3.06, 95%CI;1.04-9.2).
Conclusion: These results suggest that MIF gene polymorphism is associated with response to glucocorticoid treatment in patients with SSNHL. This might indicate the involvement of various autoimmune and non-autoimmune mechanisms in the pathogenesis of the disease and the role of MIF gene in patients with autoimmune SSNHL with better response to glucocorticoid treatment which requires further investigation.

J02.03
Association between genetic polymorphisms of NQO1 C609T with risk of Cataract

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Introduction: Cataract is multifactorial eye disease that identified with disturbance of transparent ocular lens. There is significant evidence that oxidative damage acts as a major factor in the initiation and progression of numerous diseases, such as Cataract. NQO1 is antioxidant enzyme that prevent cells from oxidative stress. The aim of the present study is to investigate the association between *NQO1 C609T* polymorphism with susceptibility to Cataract.
Material and Method: We here carried out a case-control study that included 190 cataract cases and 190 healthy subjects. We examined the genotype distribution of *NQO1 C609T* (Pro189Ser) polymorphism, using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) approach, to investigate the possible role of this SNP as a risk factor in Cataract development.
Result: We found that the variant CT heterozygous and CT/TT genotypes of the *NQO1 C609T* polymorphism were associated with increased risk of Cataract (CT vs. CC: OR = 1.61, 95% CI = 1.02-2.52, p value= 0.038), (CT/TT vs. CC: OR = 1.56, 95%CI =1.02-2.4, p value= 0.04).
conclusion: Current study showed that there is significant relationship between Cataract and *NQO1 C609T* polymorphism. With respect that NQO1 enzyme has a role in detoxification so decreasing its function by changing Pro189 to Ser increase the risk of Cataract.

J02.04
Molecular diagnostics of Pendred syndrome and Enlarged Vestibular Aqueduct in Russian patients.

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Pendred syndrome is autosomal recessive inherited disorder characterized by a combination of sensorineural hearing impairment and euthyroid goiter, it accounts for 7.5% of all deafness. Hearing impairment is usually accompanied by abnormal development of the bony labyrinth (Enlarged vestibular aqueduct - EVA and / or Mondini dysplasia). Mutations in the *SLC26A4* gene, which codes for the pendrin protein, are responsible for both of Pendred syndrome and EVA, or Mondini dysplasia.
Prevalence and etiology of Pendred syndrome and / or EVA in Russian patients have not been studied. In the course of this work was studied a DNA samples of 10 patients with Pendred syndrome and / or EVA by using Sanger sequencing of entire coding *SLC26A4* gene sequence. The analysis of two patients with Pendred syndrome and one patient with EVA detected biallelic mutations in *SLC26A4* gene, in other patients mutations were not found. It was found, that no frequent specific *SLC26A4* pathogenic variants are present in the samples, one revealed mutation c.222G>T (p.Trp74Cys) is not published earlier. In connection with the results obtained, we started to research mutations in the *SLC26A4* gene in patients with Mondini dysplasia.

J02.05
Tumor Necrosis Factor promoter polymorphisms in Romanian patients with Age-related Macular Degeneration. Results from a pilot-study

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Age-related Macular Degeneration (AMD) is condition that usually affects older adults, finally leading to blindness. The risk factors for AMD include genetic background. TNF-alpha polymorphisms were studied mostly in Asian populations regarding their influence on susceptibility of AMD (1).
We aimed to investigate two single nucleotide polymorphisms (SNP) of TNF-α in AMD patients from Romania regarding their possible influence on their clinical characteristics. We assessed 275 subjects (67 AMD patients versus 208 healthy controls) for -308 G/A (rs1800629) and -857C/T (rs1799724) TNF-alpha polymorphisms. These SPSs were genotyped using Real Time PCR technique (Taqman SNP Genotyping Assays C_2215707_10 and C_7514879_10 respectively, Applied Biosystems, USA). Statistical analyses, including Hardy-Weinberg equilibrium (HWE), were performed by using the SNPStats web tool for the analysis of association studies; values ≤0.05 were considered significant.
The distribution of both TNF-α polymorphisms were in HWE in all studied groups. The frequencies of minor alleles -308A and -857T and were similar in AMD patients and controls (0.07/0.12 and 0.25/0.20, respectively). The investigated SNPs show no significant difference regarding the allele carriage or genotype frequency between AMD and control subjects. Three main haplotypes were constructed (-308G/-857C, -308G/- 857T and -308A/-857C). There was found no significant association between any of these haplotype and AMD (global interaction p-value = 0.22).
Conclusion: TNF-alpha polymorphisms (-857 C/T and -308 G/A) seems to not influence susceptibility of AMD. These results should be confirmed on larger patients' cohort.
Bibliography:
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J02.06

Mutation analysis of the PAX6 gene in congenital isolated aniridia patients from Russia

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Aniridia (OMIM 106210) is a rare congenital ocular developmental abnormality resulting in incomplete formation of iris as the most manifest disease feature. Aniridia is caused by mutations in paired box gene 6 (PAX6; OMIM *607108; 11p13).
Aim: mutation analysis of thirty patients with congenital isolated aniridia from Russia.
Methods: thirty patients from twenty five unrelated families undergo ophthalmic examination and DNA-testing, which includes initial direct sequencing of PAX6 gene fourteen exons and after MLPA analysis (MRC Holland SALSA MLPA probmix P219-B2 PAX6, Amsterdam, the Netherlands). Subsequent linkage analysis validates revealed large deletions.
Results: There are nine patients with family history and twenty one sporadic cases.
Missense, nonsense, splice change, deletion/insertion mutations, small insertions and deletions were identified by sequencing in nineteen out of twenty nine cases. Revealed six novel point mutations are p.Asn164ThrfsTer174, p.Ser349ArgfsTer39, p.Ser294Val fsTer71, p.Ala99Leu fsTer54, p.Glu7Arg, p.Gln171Ter; seven known mutations are c.1032+6T>G, c. -128-2delA, p.Gln47Arg, p.Arg103Ter, p.Arg203Ter, p.Arg240Ter, p.Arg261Ter. Large deletions are determined in ten cases (a third of all identified changes, and a half of de novo).
Three unrelated families have nonsense mutation p.Arg203Ter, that is one of the known and commonly defined mutations in diverse populations. Likewise in three unrelated cases we identified a frequent known large deletion chr11:31307603_31650221del. Thus we have determined twenty one different mutations. In one case we fail to reveal either PAX6 mutation or lack of adjacent chromosome11region.
Five novel truncating mutations and a high rate of de novo defects including large rearrangements make up the peculiarity of mutations spectrum in patients from Russia.
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J02.07

Analysis of associated with hearing loss GJB2 and GJB6 mutations in Cherkessk urban population of the Republic of Karachay-Cherkessia (Russia)

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Medical and population genetic study of nonsyndromic hearing loss (NSHL) is done in Cherkessk, an urban settlement in the Republic of Karachay-Cherkessia (Russia) (RKC). Connexins genes GJB2 and GJB6 are the most relevant in NSHL etiology. Up to date more than 200 pathogenic mutations associated with NSHL and numerous polymorphisms with unclear clinical consequences are detected in the GJB2 gene. In this study 87 RKC patients with NSHL have been examined for GJB2 coding region mutations and two GJB6 gene large deletions: DEL (GJB6-D13S1830) and DEL (GJB6- D13S1854).
24 RKC patients are homozygous and 7 - heterozygous for 35delG mutation in the GJB2 gene. The frequency of the 35delG mutation in total patients sample is 31.61%. The mutation 313del14 is revealed in NSHL patients from RKC (1.19%; n=2). Four sequencing variants noted as neutral polymorphisms are detected: c.79G>A (n=2), c.341G>A (n=1), c.186C > T (n=1) and c.457G>A (n=1). Pathogenic significance of the change c.457G>A is ambiguous (Matos T.D., 2011). In one case the polymorphic variants c.341G>A and c.79G>A compose a known haplotype. Neither but the 35delG mutation in the GJB2 gene, nor two large deletions in the GJB6 gene are detected. 16 out of 87 NSHL patients are Karachays. Only one Karachay patient has the mutation 35delG in homozygousity (6.25%). Screening of 136 healthy Karachays from the urban settlement Cherkessk (272 chromosomes) reveals one heterozygous carrier of the 35delG mutation in the GJB2 gene. Low population frequency of this mutation (0,368%) correspondes to low frequency among Karachay NSHL patients.
This work was partially funded by RFBR grants 14-04-00525 and 15-04-01859

J02.08

Long-term observation of patients with hereditary eye pathology

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Objective. Many systemic genetic diseases and syndromes e.g. Marfan syndrome, MPS disorders, chromosomal abnormality syndromes have significant ocular pathology. Whilst rare individually, together these disorders are a significant cause of blindness and visual impairment.
Sometimes ocular sings are the first manifestation of the hereditary diseases. Here we report on long-term observation of the patients with eye pathology.
Material and methods. There were 25,814 patients aged from 1mn to 30 yr under our observation from 1985 till 2015. Routine basic ophthalmic observation was performed for all patients. Genetic tests were used for patients suffering from hereditary diseases and their relatives.
Results. 405 patients (1.57%) were found to suffer from hereditary diseases with ocular symptoms. There were monogenic AD diseases: Best disease (n-30), Marfan syndrome (5), Waardenburg syndrome (n-12), Goldenhar syndrome (n-6), Crouzon syndrome (n-2), Pringle-Bourneville disease (n-1), Rieger syndrome (n-1), Sturge-Weber-Krabbe syndrome (n-3); AR diseases: albinism (59.3%), Usher syndrome (n-24), Ehlers-Danlos disease type VI (n-1), Goldmann-Favre syndrome (n-3), Stargardt syndrome (n-10); Bloch-Sulzberger syndrome, XD (n-5); lysosomal storage diseases: GM2 gangliosidosis Tay-Sachs (AR, n-3) and Sandhoff (AR, n-2), MPS1(AR, n-1), MPSIV (AR, n-1), neuronal ceroid lipofuscinoses Jansky-Bielschowsky (AR, n-1), Batten-Spielmeyer-Vogt (AR, n-1). 15 patients suffed from retinitis pigmentosa Leber. 37 patients were diagnosed with Down disease. Embryopathy was represented Gregg syndrome (n-1). Using genealogical analysis some relatives of the probands were found to have precursory or latent symptoms of eye disturbances
Conclusions. Early ophthalmic and genetic examination, specific treatment were the necessary condition for function optimization and social adaptation.

J02.09

The analysis of the CRYAA gene in patients with hereditary congenital cataract from Bashkortostan Republic (Russia)

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Introduction: Cataracts are one of the leading causes of blindness in humans. To date, there are 22 genes, mutations in which are responsible for congenital cataract development. Mutations in the αA-crystallin (CRYAA) gene are one of the most frequent causes of congenital cataract. Different mutations in these gene lead to the development of distinct cataract phenotypes. The aim of the study was to analyze the CRYAA gene in patients from Bashkortostan Republic affected with congenital cataract.
Objective: DNA samples of 40 unrelated patients with isolated form of hereditary congenital cataract from Bashkortostan Republic were analyzed.
Methodology: The analysis was performed by direct sequencing of coding regions of the CRYAA gene.
Results: In three unrelated families we identified two new mutations which hadn't been described previously - c.253C>T (p.Leu85Phe) and c.291C>G (p.His97Gln). In a family with p.Leu85Phe mutation we found autosomal dominant congenital nuclear cataract associated with microphthalmia and microcornea, as well as strabismus and nystagmus developed later. Patients from two families with p.His97Gln mutation demonstrated isolated form of autosomal dominant congenital nuclear cataract. The total contribution of CRYAA gene mutations to the development of congenital hereditary cataract in the investigated region was 7.5 %.
Conclusion: Thus, two previously undescribed structural changes in the gene CRYAA were detected in hereditary congenital cataract patients from Bashkortostan Republic. To determine their functional significance further investigations are required. The study was supported by RFBR grant (14-04-97007_r_povolgie_a).

J02.10

RP1 gene polymorphisms in a Retinitis pigmentosa patient affected by Congenital adrenal hyperplasia: a genotype - phenotype correlation

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Introduction: Retinitis pigmentosa (RP) **(OMIM #268000)** is a genetic di-

sease involving the retina, the eye back portion, photosensitive and appointed to focus light signals towards the optical nerve and brain, after phototransduction. It is an uncommon condition affecting about 1/4,000 people in the USA and 1-5/10,000 in Italy. The term "pigmentosa" deals with the characteristic appearance of abnormal areas of pigment into the retina. Degeneration induces a slow and progressive death in photoreceptors and retinal pigment epithelium, losing ability to transmit brain the visual informations. Today about 50 RP causative genes are known[1]. We focused our attention on RP1, encoding a microtubule associated protein (MAP) needed for molecule trafficking between inner and outer segment of rods. We chose this gene due to its pathways correlations with congenital adrenal hyperplasia (CAH) ones, pathology that affects the proband of a Sicilian RP affected family (9 members).
We carried out a case-control study regarding this family vs a control group of 200 healthy sicilian donors.
The association of 5 single nucleotide polymorphisms (SNPs) of RP1 exon 4 and its 3' UTR with RP family phenotype was investigated .
Material and Methods: Genotyping was performed by PCR-RFLP (Polymerase-Chain-Reaction- Restriction-Fragment-Length-Polymorphism) and direct sequencing.
Results and Conclusions: Data obtained highlight the fundamental role of a haplotype, inferred by all analyzed RP1 polymorphisms suggesting a possible genotype-phenotype correlation between these SNPs coexistence and query disease.
[1] Zhao L. et al, Next-generation sequencing-based molecular diagnosis of 82 retinitis pigmentosa probands from Northern Ireland, *Hum Genet*. 2015; **134**(2):217-30

J02.11
GJB2 and GJB6 gene testing and genetic counseling in Romanian persons with non-syndromic hearing loss

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Hearing loss is one of the most common deficiencies at birth and is the most common disorder worldwide. One in about 500 newborns has permanent bilateral deafness. Epidemiological studies have shown that 50-60% of deafness cases have genetically causes and the mutations at the DFNB1 locus containing two genes - GJB2 and GJB6 - account for over half of non-syndromic hearing loss cases. Thus, our aim is to provide an updated spectrum of the GJB2 and GJB6 genes mutations in Romanian population in order to ensure an adequate approach.
Our studies (350 normal hearing persons and 125 children with non-syndromic hearing loss) based on molecular techniques (ARMS-PCR, PCR multiplex and DNA sequencing) found a carrier rate of 3.14 in Romanian population for 35delG GJB2 gene mutation. Analysis of the GJB2 gene revealed that about 50% of hearing impaired children presented 35delG mutation. The second mutant allele in the GJB2 gene was c.71G>A found in homo- or heterozygous forms as well, followed by c.-23+1G>A and c.380G>A mutations with lower frequencies. Also we found that the mutations in the GJB6 gene are not significant for Romanian population.
Considering all these aspects, genetic counseling should be offered in case of marriages between individuals with GJB2 mutations in their families. The genetic testing has also biomedical implications and defines new guidelines for clinical practice. Neonatal screening could provide an early diagnosis allowing appropriate intervention and increasing the chances of recovery of hearing in children.
This work received financial support through the project „CERO - Career profile: Romanian Researcher", grant POSDRU/159/1.5/S/135760.

J02.12
Mutation detection of *PDS* gene in Iranian patients with hearing loss

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Introduction: ***PDS*** (*SLC26A4*) has been mapped on 7q22-31.1 and is the second most common gene accounting for hereditary hearing loss (HL), after *GJB2*. The product of *PDS* is pendrin, a transmembrane anion transporter. Mutations of *PDS* are associated with recessive non-syndromic HL (DFNB4) and Pendred syndrome (PS). To date only a little is known about *PDS* mutations in Iran. In the present study mutations of this gene were investigated in Iranian subjects with HL to identify prevalence and types of *PDS* mutations in these patients.
Methods: Thirty unrelated Iranian families with hereditary hearing loss and without *GJB2* mutations were investigated for linkage to DFNB4 using 4 short tandem repeat (STR) markers. Following that, DNA sequencing of all 21 exons, their adjacent intronic sequences and promoter of *PDS* was carried out for mutation detection in families showed linkage. Clinical phenotype of patients with mutation was studied with temporal bone CT- scan, thyroid ultrasonography and thyroid hormone assays.
Results: Three families were linked to DFNB4 locus. Sequencing was clarified 4 *PDS* mutations (c.65-66insT, c.2106delG, c.863-864insT, c.881-882delAC) in exons 2, 19 and 7. Clinical phenotype investigations showed PS in these three families.
Conclusion: This study reveals the importance and specificity of *PDS* mutations in Iranian patients with hearing loss. So screening of this gene is important in hearing loss molecular diagnostics. Morever PS is probably a common syndrome among Iranian deaf patients.

J02.13
Panel base on next-generation sequencing for mutation detection in hearing impairment

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Hearing loss is the most common sensory impairment of human. At least 50% to 60% of childhood hearing loss was inherited. Identifying the genetic basis of deafness is a critical procedure for the clinical evaluation of deaf persons and their families. To date, 136 deafness loci have been identified, and about 90 genes have been cloned. However, the mutation frequency of many genes for HL is not clear, and the causes for majority deafness patients remained unknown. It is critical to develop an effective, comprehensive genetic testing platform for hearing loss.
We designed a panel including 307 deafness genes and the chondriogene, 76 samples with identified deafness gene mutations and YH genome DNA sample were used for the validation. And then screenings were carried out for a total of 147 patients with hearing loss, by target-capture and NGS.
For the 77 validation samples, the average depth of the targeted regions was more than 250 fold. The sensitivity and specificity for the mutation detection was 100%.
For patients with non-syndromic hearing loss, the positive rates were 56.6% (56/99) for early-onset hearing loss and 39.5% (17/43) for late-onset deafness, and candidate mutations were identified in 2 out of 5 patients with syndromic hearing loss.
The results show that our platform is sufficient accurate and effective for molecular diagnosis of hearing impairment.

J02.14
Is the KDR gene associated with Central serous chorioretinopathy

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Central serous chorioretinopathy is an idiopathic ocular defect occurs in serous tissue of the macular region. Studies, showed that intraocular hemorrhage and fluid accumulation lead to reduced vision. Vascular endothelial growth factors (VEGFs) causes to many retinal diseases via inducing angiogenesis and leads to macular edema by increasing vascular permeabilities. TheKDR gene, is a VEGF receptor gene. Given that the VEGF gene family structure and cellular mechanisms, KDR gene mutations have been associated with central serous chorioretinopathy detachment.There has not been any genetic studies about KDR gene related with CSCR. Previousstudies indicates that VEGF-A plays a crucial role in physiopathology of CSCR disease.Therefore we have identified KDR gene sequence as candidate gene and region to explain potential effects of genetic factors on CSCR.
The aim of our project is to investigate the mutation spectrum in the KDR gene in patients with central serous chorioretinopathy and to determine the genotypephenotype relationship. Exonic regions of the KDR gene were sequenced with Sanger method to 32 cases with CSCR and 32 control groups. Consequently known rs140825421 (p>0.05) and rs2305948 (p=0,3) missense mutations, rs35961234 (p>0.05) and rs77722107 (p>0.05) synonymous, rs2219471 (p=0,59) and rs2305949 (p=0,86) intronic, rs4421048 UTR mutation and one novel hetdel_TAA deletion (p>0,05) were found.
Our results, were the first genetic study of KDR gene mutations causing CSCR. Emphasizes the requirement for complete screening of the mRNA sequence of KDR and VEGF-A genes for molecular diagnosis. The identification of mutations in genes of interest would also have importance in diagnosis and genetic counseling in our population

J02.15

Molecular genetic analysis of family with autosomal dominant deafness from Tatarstan revealed a novel mutation in MYO7A

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Introduction: Genetic epidemiological examination of isolated populations can often reveal relative abundance of hereditary conditions, which are rare in other populations. Rural regions of Tatarstan (Russia) are characterized by high occurrence of isolated hereditary hearing loss. Here we describe a family with recurrent postlingual hearing loss: the proband (woman) and one of her four sisters have hearing loss that manifested postlingually. Affected sister of proband has two children; one of them also developed progressive deafness. Molecular diagnosis in the family was determined with the aid of whole exome sequencing (WES) in conjunction with clinical observations.
Materials and Methods: During the field expeditions in Tatarstan, medical genetic examination and peripheral blood collection of familial cases of hearing loss was performed. Isolated DNA was routinely screened for frequent pathogenic mutations in *GJB2* gene. WES was performed on Illumina HiSeq 2000 at 85× depth. Verification of mutations identified by WES was done by Sanger sequencing.
Results: Screening for frequent mutations failed to identify common cause of observed condition. Among 60 genes associated with hearing loss, WES revealed an undescribed heterozygous mutation p.R848Q in *MYO7A* gene, located at a highly conserved position. The known clinical presentations and mode of inheritance described for *MYO7A*-related hearing loss corresponded well to our case.
Conclusions: Combination of WES with detailed analysis of clinical data enabled to identify causative mutation in a family with autosomal dominant hearing loss. This approach can enhance the current diagnostic pipeline for this condition.
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J02.16

Targeted next generation sequencing successfully diagnoses 'rare' metabolic disorders for which childhood cataract is an early indication

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Introduction
Childhood cataract (CC) is the leading global cause of lifelong visual loss. Around 50% of cases have a genetic aetiology. Inborn errors of metabolism represent a subset of causes, a number of which are treatable, making early identification crucial. Extreme genetic and phenotypic heterogeneity impedes the traditionally complex clinical/genetic/biochemical diagnostic pathway. Next generation sequencing (NGS) technologies have transformed the diagnostic approach.
Methods
We have successfully implemented an NGS target enrichment to screen 115 CC-associated genes. A close collaborative effort has enabled the development of an efficient diagnostic pipeline linking detailed clinical phenotyping, high through-put sequencing, data storage, bioinformatics and scientific interpretation to create a linear care pathway from patient to report.
Results
Cataract-targeted NGS has enabled a diagnosis rate of 75% in CC patients; 18% of cases are attributable to metabolic disease, indicating a higher prevalence than the literature suggests. Six different inborn errors of metabolism were identified, including apparently rare conditions such as stomatin-deficient cryohydrocytosis, lathosterolosis and cerebrotendinous xanthomatosis.
Discussion
Cataract-targeted NGS streamlines the care pathway of CC patients, reducing the requirement for a battery of diagnostic tests habitual to the pre-genomic era. Precise diagnosis of metabolic conditions for which CC is a primary indication, permits i) initiation of early therapeutic intervention to prevent disease progression ii) delineation of rare/complex phenotypes and iii) greatly improves diagnostic outcomes attributable to the implementation of stratified genomic medicine.

J02.17

RGS6: A novel gene associated with congenital cataract, intellectual disability, and microcephaly in a Tunisian family

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Introduction: Congenital cataracts show considerable clinical and locus heterogeneity. About 39 loci are involved in isolated or primary cataracts. Congenital cataract can occur in association with a large number of different metabolic diseases or genetic anomalies including intellectual disability and microcephaly. Here is the first report of gene identification in inherited congenital cataracts associated only with intellectual disability and microcephaly.
Materials and Methods: Five members (3 normal, 2 affected) of a 4-generation consanguineous Tunisian family with autosomal recessive congenital cataract associated with intellectual disability and microcephaly were screened. A whole-genome scan was performed with polymorphic microsatellites and homozygous regions were analyzed with integrated Systems Tool for Eye gene Discovery (iSyTE). Selected genes were analyzed by direct sequencing.
Results: Using whole-genome scanning, we identified six runs of homozygosity shared among affected members. Analysis of these regions by iSyTE allowed us to select 3 genes (*RGS6*, *PCNX*, and *P4HA1*) according to their expression in 3 critical stages of lens development. Upon screening for mutations by sequencing analysis, we found a novel mutation in RGS6, the splice-acceptor variant c.1369-1G>C that was not previously reported in congenital cataract phenotypes. Bioinformatics analysis suggested a deleterious effect of this mutation on protein structure and function.
Conclusion: This is the first report of a splice-site mutation of a novel lens specific gene *RGS6* in a phenotype associating AR congenital cataract, intellectual disability and microcephaly. This study highlighted the genetic heterogeneity of congenital cataract.

J03.01

Prenatal diagnosis in families with autosomal recessive polycystic kidney disease - case reports

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Autosomal recessive polycystic kidney disease (ARPKD) is a severe form of polycystic kidney disease characterized by fusiform dilatations of renal collecting ducts and ductal plate malformation causing biliary ectasia and hepatic fibrosis. The most severely affected fetuses display enlarged echogenic kidneys, oligohydramnios and pulmonary hypoplasia. Arterial hypertension affects up to 80% of children with ARPKD and usually develops within the first months of life. ARPKD is caused by mutations in the PKHD1 gene located on chromosome 6p12 and extending over 470 kb of genomic sequence. The severity and early manifestation of ARPKD make parents of affected child seek reliable prenatal diagnosis to guide future family planning. Even though the standard second-trimester US imaging is still state-of-the-art method of ARPKD diagnosis, it is often not early enough for pregnancy termination. For that reason early prenatal diagnosis is feasible only by molecular genetic analysis. Here we present 2 Czech families with severe form of ARPKD causing perinatal death of index patient. In these families amplicon-based next-generation sequencing and MLPA (multiplex ligation-dependent probe amplification) analyses of the PKHD1 gene in fatally affected child were performed and so facilitated quick prenatal genetic diagnosis in next pregnancy when only positive amplicons with found mutation were analyzed, and therefore helped the diagnosis of ARPKD based most importantly on ultrasound screening. In both families only one mutation was confirmed in fetus and healthy baby was born.
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J03.02

The prevalence of iceA Helicobacter pylori gene in Eastern Siberia (Sakha Republic)

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Helicobacter pylori (Hp) is associated with gastrointestinal diseases [Suerbaum et al., 2002]. The iceA gene is the main virulence factor [Atherton et al., 1995] which most associated with peptic ulcer and chronic gastritis (CG). Recently, study was undertaken on a meta-analysis of clinical outcomes in carriers of different variants of iceA, which has been studied more than 5,000 people and showed that the majority of patients iceA1 allele is associated with peptic ulcer [Shiota et al., 2012]. Similar researches have not been conducted in Eastern Siberia (Yakutia). The aim of this study is to examine the prevalence of iceA Hp in Yakutia. The studied group consisted DNA samples, which were isolated from biopsies of 42 Yakut patients with confirmed diagnosis of CG by histological and cytological study. Among the investigated 42 clinical isolates iceA1 and iceA2 were detected in 33 and 9 patients, respectively. The prevalence of iceA1 in Yakutia was 78.5% (CI=0.64-0.88), which was significantly higher than in Europe - 29.5% (CI=0.34-0.44) and America - 46.5% (CI=0.31-0.41), but not differed from Asia - 58.9% (CI=0.58-0.64). The prevalence of iceA2 in Yakutia was 20.9% (CI=0.11-0.36), lower than in America - 55.4% (CI=0.50-0.60) and in Europe - 38.8% (CI=0.38-0.48), but not different from Asia - 26.3% (CI=0.22-0.28). Thus, the prevalence of iceA1/A2 in our study significantly differed from the prevalence in Europe and America ($p<0.05$), but was close to the values in Asian populations ($p>0.05$).

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J03.03

Biochemical And Genetic Markers Of Autoimmunity Of Hashimoto's Thyroiditis

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Hashimoto's thyroiditis (HT) is one of the most widely-spread diseases of the thyroid gland. AITDs are highly prevalent, affecting 1% to 5% of the population worldwide. One of the proposed mechanisms of the HT formation bases on genetic defect of specific CD8+ T-Lymphocytes infiltration of thyroid tissues and progressive thyroid cells damage. Genome-wide association syudies have revealed about 15 genes associated with HT such as MHC (HLA II region), CTLA-4, PTPN22, some proinflammatory cytokines (IL-1B, IL-4 and IL-6), TNF-a and TNFRSF1A, TNFRSF1B.

Subjects. The study included 137 unrelated healthy women who had no family history of chronic autoimmune, cardiovascular and inflammatory diseases, and 161 women with HT with normal and decreased thyroid function (eu- and hypothyroid respectively).Immunophenotyping of lymphocytes performed by flow cytometry «FACSCalibur». Determining the level of annexin V, TNF-a, TRAIL, and the serum was performed by ELISA using commercially available kits according to the manufacturer's instructions («Bender MedSystem», Vienna. Austria).

Genotyping of rs231775, rs5742909, rs4553808 gene CTLA- 4, rs2476601 gene PTPN22, rsl143634 gene IL 1B rs2243250 IL-4 gene, rsl800795 gene IL-6, rs1800629 gene TNF-a, rs4149570 and rs800692 TNFRSF1A gene,rs1061622 and del 15 bp gene TNFRSF1B was performed by CFX-96 real-time amplification system (BioRad, USA).

Results:

1. Hashimoto's thyroiditis characterized by TRAIL- induced apoptosis.
2. The genetic markers of HT are polymorphic loci rs4553808 and of rs231775 gene CTLA-4; rs1800629 gene TNF-a, rs800692 gene TNFRS1A; rs1143634 gene IL-1B.

Genes polymorphisms associated with Hashimoto's thyroiditis

Polymorphism	Predisposing		Protective	
	Genotypes	OR	Genotypes	OR
rs4553808 CTLA-4	AG	1,83	-	-
rs231775 CTLA-4	GG	4,04	AA	0,34
rs1800629 TNF-a	AG/GG	3,52/10,8	-	-
rs800692 TNFRSF1A	CC	5,04	CT/TT	0,25/0,19
del 15 bp TNFRSF1B	DD	1,41	ID	0,35
rs1143634 IL-1β	CT	1,88	TT	0,15

J03.04

X-linked Alport syndrome: cardiovascular symptoms and affected females.

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Background: Alport syndrome (AS) is an inherited disease. It is expected, that X-linked AS affected only males. In patients with autosomal dominant and autosomal recessive AS both sex affected.

Methods: coding exons and splice sites of the abovementioned genes were sequenced in on Ion Torrent platform.

Results: in Russian family were revealed 1 affected male and 4 affected females with a dominant missense mutation c.G3098A, p.1033 G>D in the collagen type IV alpha-5 gene causes X-linked AS. New phenotype findings were: cardiovascular abnormalities (asymptomatic aortic root enlargement, aortic and mitral valves insufficiency in affected male; ascending aorta enlargement, aortic and mitral valves insufficiency in 63 years old female; mitral valve insufficiency in 5 years old girl; early onset of systemic hypertension in 1 male and 3 females; early onset (before 3 years of age) and progression of proteinuria up to 2,0 g/l in affected females; absents of any ocular abnormalities. Glomerular filtration rate was low in affected male - 50 ml/min and slightly abnormal in all but one affected female: in 63 years old woman - 78 ml/min, in 29 years old woman - 82 ml/min, in 5 years old girl - 88 ml/min.

Conclusion: Affected females with X-linked AS demonstrate clinical symptoms of kidney disease and cardiovascular abnormalities. Cardiology examination may be necessary in all affected subjects.

J03.05

Correlation of SNP polymorphisms in modifier genes with phenotype of Slovak cystic fibrosis (CF) patients

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Cystic fibrosis (CF) is the common lethal autosomal recessive disorder in whites. Classically, individuals with CF develop a disease characterized by progressive bronchiectatic lung disease with bacterial infections, pancreatic insufficiency, meconium ileus, chronic sinusitis and male infertility.

Even in patients with CF with identical CFTR genotypes, there is a wide range in the severity of lung disease. Environment and polymorphism in other genes play a particularly important role that modify the phenotypic presentation of a disease. So far, more than 80 modifying genes have been described in CF from those we have selected 11 that may influence the phenotype of patients with CF, specifically to lung disease, immunity, nutrition and liver cirrhosis. Among the selected genes include MBL2 (rs5030737, rs1800451, rs1800450 and in promoter -220), SERPINA1 (rs28929474, rs17580), ACE (rs4646994), TGFβ1 (rs1800470, rs1800471), TNFα (rs1799724, rs1800629, rs361525) GSTP (rs947894), GSTM (in-del), GSTT (in-del), HFE (rs1799945, rs1800562, rs1800730), NOS1 („AAT" repetition in intron13 and 20) and ADRβ2 (rs1042713, rs1042714, rs1042717). DNA polymorphisms were typed by PCR reaction, directed sequencing, single base primer extension assay (SNaPshot) and fragment analysis in 196 patients and 96 controls. Also spirometric, microbiological and other clinical data were collected from medical records.

The main aim of the study was the description the correlation of gained genotypes in modifying genes to the phenotype (clinical symptoms) of CF patients.

J03.06

The role of the cytokine genes polymorphisms in peptic ulcer disease predisposition in Volga-Ural region of Russian Federation

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Peptic ulcer (PUD) - a chronic disease based on recurrent gastric or duodenal ulcer (DU). One of the probable causes of the PUD development is infection by the bacterium Helicobacter pylori, but ulceration depends on a large number of endogenous and exogenous risk factors. The aim of this study was to analysis the association of cytokine genes polymorphisms

IL1B (rs1143634, rs16944), IL1RA (rs71941886), IL6 (rs1800796), IL8 (rs4073, rs2227307), IL10 (rs1800872) and TNFA (rs1800629). This study enrolled 353 patients with gastric and duodenal ulcers, 114 of them were infected by H.Pylori, the control group included 285 unrelated individuals without gastro-duodenal pathology with different ethnic origins (Russians, Tatars, Bashkirs). Genotyping was performed by TagMan real-time PCR. The analysis has revealed a strong association of rs1143634*C allele and rs1143634*C/C genotype of IL1B gene with the risk of PUD in Bashkirs (p = 0.006; OR = 2.87 and p = 0.002; OR = 4.49, respectively), with the risk of duodenal ulcer (p = 0.01; OR = 1.46 and p = 0.009; OR = 1.64, respectively). We have also detected that frequency of A/A genotype of the rs4073 of the IL8 gene are significant more prevalent in healthy donors than in H.Pylori-positive individuals (p = 0.02; OR = 0.46). The study shows that the polymorphisms s of IL1B, IL8 genes may contribute to the structure of the genetic predisposition of PUD in Volga-Ural region of Russian Federation.

J03.07

Importance of HLA typing in diagnosing celiac disease in men and women

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Introduction: Chronic gluten intolerance (celiac disease) occurs in genetically predisposed individuals. It is a multifactorial disease, which is affected by several environmental, immunological and genetic factors. The main factor affecting the genetic predisposition for celiac disease is HLA II. class DQ2 and DQ8.
Materials and Methods: In the group of patients, there were 200 women and 80 men (average = 38,5 years), who were examined for the HLA system II. class, heterodimers HLA DQ2 and HLA DQ8. To identify major haplotypes in the context of celiac disease, we used the method of Reverse Dot Blot with the aid of a commercial kit CeliacStrip (Opegen).
Results: The frequency of the haplotype HLA-DQ2 cis in women was 20.30% and 12.14% in men in any given group of patients. Women with DQ2 trans Hp2 were 12.38% and 7.6% men. In the statistical evaluation of the patients we did not find statistically significant differences in relation to gender, among tested men and women.
Conclusion: In comparison to the risk of haplotypes in women and men in this group of patients, it was found that women have a slightly higher prevalence for the risk of haplotype HLA DQ2.5, HLA DQ2.2 also combinations of HLA DQ2.5 and HLA DQ8 compared to men. Men have a higher frequency of risk due to the presence of haplotype HLA DQ8.
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J03.08

Melatonin Receptor 1b Polymorphisms In Bulgarian Patients With Policystic Ovary Syndrome.

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Background: Polycystic ovarian syndrome (PCOS) is a complex endocrine disorder with a pronounced genetic predisposition. Pineal hormone melatonin could modulate reproduction pattern in several animal species, and disturbed melatonin secretion has been described in women with hyperandrogenic states. Nevertheless, the role of melatonin receptor polymorphisms for the development of PCOS is poorly investigated. Therefore, the present study aimed to investigate the relationships between melatonin receptor 1B (MTNR1B) polymorphisms rs10830962 and rs10830963 and clinical features of the syndrome.
Materials and methods: Genotyping for MTNR1B rs10830962 and rs10830963 was performed in 59 patients with PCOS by PCR-RFLP analysis.
Results: MTNR1B rs10830963 G allele was related to elevated levels of DHEAS in the investigated patients (p=0.007), while no significant associations with testosterone levels, presence of hirsutism or obesity were found (p>0.05). MTNR1B rs10830963 G allele was associated with a significantly lower LH to FSH ratio in obese PCOS patients (p=0.026). No significant associations between MTNR1B rs10830962 and PCOS clinical or hormonal features were established (p>0.05).
Conclusions:The present results suggest that MTNR1B rs10830963 but not rs10830962 polymorphism could influence the clinical expression of PCOS in women.
The present study was financially supported by the Medical University - Sofia.

J03.09

Silencing of Important Molecules Having Roles in Pathogenesis of Idiopathic Pulmonary Fibrosis via RNA interference and Development of New Therapeutic Modalities

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Idiopathic Pulmonary Fibrosis (IPF) is a disease that is characterized by the deposition of an excessive degree of myofibroblast cells and extracellular matrix components in the lower respiratory tract and lung interstitium. Median survival is 3 years after initial diagnosis. Prevalence rate varies from 14 to 43 per 100 000 people. Today, it is thought that recurrent epithelial damage and aberrant wound healing are the basis of IPF pathogenesis, resulting in the accumulation of fibroblasts in the lung. In addition, coagulation, apoptosis, angiogenesis pathway disorders, oxidative damage, and most recently epithelial–mesenchymal transition (EMT) are implicated in the pathogenesis of this disease. Major aim of our study is to show the fundamental role of osteopontin, Twist and Wnt-5a genes in IPF pathogenesis which these genes have been implicated by several studies in EMT and to show whether the suppression of these genes could be effective for IPF treatment examined. First of all, we treated A549 cell line with different dose of TGF-β to create EMT. We showed that after TGF-β treatment, E-cadherin expression is markedly decreased, on the contrary, Vimentin expression increased showing EMT. Then, Changes in the expression of genes responsible for EMT formation and fibrosis examined at the level of mRNA and protein performing siRNA knockdown for Osteopontin, Twist and Wnt-5a gene transcripts in lung alveolar cell lines.

J03.10

Socs3 Polymorphisms in childhood obesity: Is the cytokine system in operation?

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Objective: Although polymorphisms in suppressor of cytokine signaling 3 (SOCS3) was reported to be related to obesity, metabolic syndrome and type 2 diabetes in various adult studies, there is a lack of data in children. In this study, we examined eight reported polymorphisms of SOCS3 in obese Turkish children with and without metabolic syndrome and compared the results with that of controls.
Methods: 148 obese and 63 age and sex matched control children were enrolled in the study. Genotyping procedure was carried out by PCR- sequencing protocole.
Results: The frequency of rs2280148 polymorphism was significantly higher in obese children with metabolic syndrome than that of the control group, whereas the frequency of rs8064821 polymorphism was significantly higher in obese children with metabolic syndrome than that of obese children without metabolic syndrome.
Conclusion: The significant association of certain SOCS3 polymorphisms with obesity parameters in both metabolic syndrome and metabolic syndrome related insulin resistance, hypertension, and fatty liver suggest that polymorphisms in this gene may play a role in the pathogenesis of metabolic syndrome and also that they can be potentially used as a marker for attenuated or agressive disease.

J03.11

Molecular diagnosis of family with two sisters with clinical manifestation of simple virilizing form of congenital adrenal hyperplasia

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Background: Congenital adrenal hyperplasia is an autosomal recessive dis-

order caused by steroid 21-hydroxylase deficiency in 90-95% of cases. Its classical simple virilizing (SV) form leads to virilization of external genitalia in newborn females and pseudoprecocious puberty in both sexes, due to reactive androgen overproduction. SV CAH is usually associated with I172N missense mutation at the CYP21A2 gene.
Method: Two sisters were diagnosed as classical SV CAH at the age 7 and 9 years respectively by standard clinical and biochemical procedures. They had virilization, and high 17OHP (>75 nmol/L) on ACTH stimulation test. Molecular detection of nine most common point CYP21A2 mutations in the patients and family members, using the PCR/ACRS method was performed. They received hydrocortisone for several years. At 35 and 37 years they were referred to the gynecologist due to oligomenorrhea, hirsutism, and infertility. After hormonal preparation IVF was performed in the older sister with no success.
Results: P30L/II72N genotype was observed in both SV patients. The I172N mutation in the heterozygote state was observed in their other two sisters, 43 and 28 years old, without clinical manifestation of CAH. Father was heterozygote for P30L mutation. The deceased mother has not been analyzed. She was probably a carrier of I172N.
Conclusion: Simple virilizing CAH appears with virilization causing oligomenorrhoea and infertility later in life. Molecular diagnosis is essential to provide the correct diagnosis and allows appropriate clinical and genetic counseling. Our findings suggesting that P30L as well as I172N caused SV CAH in these patients.

J03.12
The incidence dynamics of congenital hypothyroidism in the Northern Russian territories during eight-year period

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Objective: To investigate the incidence of the primary congenital hypothyroidism (CH) in the Khanty-Mansiysk Autonomous Region (KHMAO) according to newborn screening (NBS) data during last eight years.
Materials and methods: Retrospective evaluation of CH incidence dynamics in newborns based on the results of NBS in 2006-2013 years was performed. TSH levels in DBS were measured using "DELFIA Neonatal hTSH" kit following evaluation with locally established cut-offs. Threshold TSH values for CH detection were >9mU/L for newborns of 4-5 days of life and >5mU/L for newborns after 14 days of life. Samples with exceeded threshold were assayed again. TSH levels 5mU/L indicated newborns at risk of CH. These children were sent to pediatric endocrinologist consultation with follow-up diagnostics and treatment. Confirmatory diagnostics was performed by serum TSH, free T4 detection. Statistical validity was assessed by Student t-test, $p<0.01$.
Results: Eight years-long NBS coverage for KHMAO region was 98.9% with TSH level evaluated in 190 589 newborns. Confirmatory diagnostics was performed in risk group of 3 563 children. 76 children were diagnosed with CH with appointed replacement therapy. Thus, CH incidence within studied territory was 1:2 508 births with statistically significant incidence increase of 4.1 times between the minimum (1:5,292 live births in 2007) and the maximum (1:1,298 live births in 2010, $p<0.001$).
Conclusions: CH incidence in KHMAO region according to NBS for the 8-year period was 1:2,508 with 98.8% coverage. Its dynamics has a statistically significant increase of 4.1 times.

J03.13
Gene - environment interactions analysis in occupational chronic bronchitis

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Occupational chronic bronchitis is one of the common disease and both genetic and environmental risk factors contribute to its etiology. A case-control study was conducted using 122 patients with occupational chronic bronchitis and 166 healthy workers to investigate the association of CYP1A2 (rs762551, rs35694136), CYP2F1 (rs11399890), NQO1 (rs1131341, rs1051740), UGT2B7 (rs7439366), CAT (rs1001179, rs769217), GSTP1 (rs1695, rs1138272) polymorphisms with the disease developing risk Analysis was performed to test for GxE interactions with exposures (smoking, PY, occupational experience) using logistic regression models.
It was shown CYP1A2 rs35694136 (P=0.02, in over-dominant model), UGT2B7 rs7439366 (P=0.002 in recessive model), CAT rs1001179 (P=0.02, in dominant model) were significantly associated with high risk of occupational chronic bronchitis development. When ethnicity, smoking, PY, occupational experience were included in the logistic regression model, it was shown association with risk of disease development for rs1131341 NQO1 (Padj=0.0004, ORadj=3.57), rs7439366 UGT2B7 (Padj=0.0024, ORadj=2.31), rs35694136 CYP1A2 (Padj=0.0041, ORadj=2.17). Statistically significant interaction with smoking status was defined for rs7439366 UGT2B7 (Pinteract=0.015, in over-dominant model) with PY-for rs11399890 CYP2F1 (Pinteract=0.05 in additive model).
Identifying GxE interaction will lead to better understanding of the development of occupational chronic bronchitis and potential biological mechanisms, and, in future, effective prevention strategies.
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J03.14
Molecular analysis of CYP21A2 gene in patients with congenital adrenal hyperplasia (CAH) by sequencing

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Congenital adrenal hyperplasia (CAH) is a hereditary metabolic disorder which is caused by the defects in the steroid 21-Hydroxylase CYP21A2 gene in an autosomal recessive manner. The most common form of this disease which is seen in more than 90% of patient is 21-Hydroxylase deficiency and 11-β- Hydroxylase placed on second stage. Identify of mutation at early stages is crucial.
AIMS:
Mutation identification in CYP21A2 gene at early stages.
Materials and Methods:
According descriptive epidemiological study patients with CAH were recruited from the Genetic Research Centre Of Tabriz in 2012 and 2013. Sampling and DNA extraction was followed by PCR on exons to find out mutation on CYP21A2 gene.Then sequencing has been done.
SPSS(version 22) was used for data analysing and descriptive statistics and the results were compared with the reference gene by blast, Generunner and MEGA-5 softwares. Obtained changes were compared with NCBI databases and also SIFT.
Polyphene and BDGP softwares was used to predict the performance of obtained changes.
Results:
prevalence of CAH in patients is in classic form (salt wasting & simple virilizing).
Analysing of the sequencing shows mutations located in Exons 6,7,8 and 10.
Study shows Q318S (g.1994 952 C>T) and V 237E (g.1383 710 T>A) were the most and E351K (g.2093 1051G>A) and V281L (g.1683 841 G>T) were the least frequent mutations. Also the level of mutation frequency in men was higher than women.
Conclusion:
Due to Observed mutations frequency in this study (was much higher than reported frequency in previous studies) Follow-up screening programs and using sequencing method for identification of mutations seems obligatory.

J03.15
A novel mutation of JAG1 causes Alagille syndrome

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Alagille syndrome (AS) is a complex multisystem disorder with manifestations primarily in liver, heart, eyes and skeleton and has also characteristic facial features. AS is caused by mutations in two genes - JAG1 (95%) and NOTCH2 (1-2%) and inherited in AD manner.
A one-month-old boy was referred to genetic counselling because of suspected Alagille syndrome. The child had cholestatic icterus and pulmonary stenosis on EchoCG. Liver biopsy was performed and it confirmed the paucity of bile ducts. The child has also other features of AS such as retinal hypopigmentation, renal dysplasia with small non-functioning right kidney and megaureter on the left side, he has malabsorption and growth failure. The family history is negative.
A mutation analysis of JAG1 gene was requested. The results revealed a previously unpublished sequence variant - an in-frame deletion c.53_73del (p.Leu18_Leu24del) in a heterozygous state. In silico analysis (HumanSplicingFinder) predicts this mutation as potentially deleterious. Sequencing of

1st exon of JAG1 gene of both parents was performed, but the deletion was not found, confirming a de novo event.
Two previously described in-frame deletions of the region which overlaps the region deleted in our patient have been reported as causative for Alagille syndrome. These are c.48_68del and c.63_74del (N.B.Spinner et al., 2000 and 2006). The sequence which is deleted in both previously reported cases and also in our case is six nucleotides long: CTGCTC and codes for one leucine and one cysteine residue. We propose that this cysteine residue plays crucial role in jagged1 protein folding, and the deletion of it affects function of the protein.

J03.16

Genotype and phenotype characteristics of patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. The first intervention study in Armenia.

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Congenital adrenal hyperplasia (CAH) is inherited autosomal recessive due to different kind of mutations in the CYP21.
Objective and hypotheses: The aim of this study was to evaluate clinical and molecular characteristics of the patients with CAH.
Metod: 20 patients diagnosed as CAH according to their clinical, hormonal and molecular finding, were in included. We thoroughly examined 20 patients with CAH and 2 asymptomatic individuals with a history of affected siblings. The mutations associated with CAH were identified by polymerase chain reaction and reverse-hybridization. The assay covers 11 mutations in the CYP21A2 gene: P30L, I2 splice (I2 G), Del 8 bp E3 (G110del8nt), I172N, Cluster E6 (I236N, V237E, M239K), V281L, L307 frameshift (F306+T), Q318X, R356W, P453S, R483P.
Result: Nine patients had classic CAH and presented with ambiguous genitalia and/or salt losing crisis. Eleven patients had the non-classic form of CAH and presented with precocious puberty. The remaining 2 subjects were asymptomatic. Screening the CYP21A2 gene, we detected Q318X heterozygous mutation in 9 patients, P30L/I2splice/del8bpE3/V281L compaund geterozygous mutation in another 2 patients, I2splice/I172N compaund heterozygous mutation in 4 patients,
Q318/R483P compaund heterozygous in 1 patient ,V281L/heterozygous mutation in 4 patients and P30L/Del8bp compaund heterozygous/I2splice hemizygous in 2 patients.
Conclusion: Larger studies are necessary: the genotype-phenotype discordance of these patients requires further explanation.

J03.17

The influence of HLA class II genes in autoimmune liver and thyroid diseases association with celiac disease in children

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Background:Recent researches proved that classical definition of celiac disease(CD) comprises only 30% of cases with genetic predisposition, the majority of patients being pauci-symptomatic.Active-case finding in groups at risk for CD is considered cost/effective.Objectives:To determine the prevalence of CD in a pediatric population with autoimmune hepatitis(AIH) and autoimmune thyroid disorders(AITD) and in control lot and to assess the clinical forms of presentation and the HLA polymorphism in all cases. Methods:We screened for CD 74 children with AITD(lot 1), 62 children with AIH(lot 2) and 60 healthy children.In patients with at least one positive autoantibody for CD, intestinal biopsy was performed.All children underwent HLA typing for DQ2/DQ8.Results:CD prevalence was 7%(5 patients) in lot 1, 6%(4 patients) in lot 2 and 0 in control lot.There weren't significant differences between the frecquency of CD cases among children with AITD and AIH(p>0,05).Most of the cases presented as silent CD(78%).All children diagnosed with CD presented DQ2/DQ8 haplotype.20% of the control subjects associated heterozygous DQ2 alleles.From 69 children with AITD/without CD, only 3 patients(4%) presented heterozygous DQ2.From 58 children with AIH and negative results for CD, 37 patients(64%) associated homo or heterozygous DQ2/DQ8 alleles.Conclusions:Recommending AITD and AIH as selection parameters for CD screening in asymptomatic children is justified by the high frequency of gluten enteropaty obtained in this study(7% and 6% respectively).CD and AIH share selected combinations of genes coding for class II HLA, which could explain their coexistence.Besides immunosuppressives, early detection and dietetic treatment of CD in AIH children may prevent progression to end-stage liver failure.

J03.18

Genealogy analysis of bronchial asthma

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Genealogical research allows to the practicing doctors to increase the effectiveness of medical and genetic counseling for secondary and tertiary prevention of the formation of groups at risk for asthma and improve mechanisms for identifying factors of manifestation.
The purpose and objectives. Purpose - genealogical analysis of susceptibility to disease relatives with varying degrees of relationship with a patient with asthma among populations of Kharkiv. Objectives: 1. Sets the coefficient of correlation between the age of manifestation of asthma in parents and children. 2. Investigate the predisposition to illness of relatives with varying degrees of relationship with a patient with asthma 3. Compare the features of sexual predisposition to asthma.
Results. Between the ages manifestation of asthma in parents and children is a direct connection. The value of the correlation coefficient for parent- children pairs is 0.54 (p <0.001). There is a variation of correlation coefficients depending on the sex of patients. Maximum correlative relationship was found in pairs „mother-descendant" (r = 0,73). In pairs „father-descendant" this index half as much. (r = 0,36). When comparing male and female predisposition to transfer asthma found that significantly more women than men who have relatives with asthma (φ♀ = 1,861, φ♂ = 1,281, Ffakt.> Ftabl.). Thus amount of sick relatives among the polled women-patients twice as much amount of sick relatives of patients-men as the first so second degree of cognation.
Conclusion: The maximum risk of asthma are relatives of the first degree relatives, particularly if the mother is sick.

J03.19

Genes involved in fibrogenesis in predisposition to type 1 diabetes and diabetic nephropathy

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Vascular complications of diabetes are the leading cause of preventable morbidity and mortality of patients with diabetes. Case-control study included 585 people Russian living in Siberia (Tomsk, Kemerovo) - 285 patients with type 1 diabetes (T1D), among which 123 had diabetic nephropathy (DN) and 300 population-based controls. We studied 58 SNPs of 48 genes that are involved in the process of fibrogenesis, responsible for the functioning of the endothelium, associated with diabetes and diseases of the cardiovascular continuum. Specimens were evaluated using the Sequenom MassARRAY (USA). Association with the disease was obtained for the following markers: MMP3 rs679620 (p=0.004), ITGB5 rs1007856 (p=0.039), ITGA4 rs1143674 (p=0.002), LIG1 rs20579 (p=0.003), ADAMDEC1 rs3765124 (p=0.014), IFNL2 rs12980602 (p=0.029), PARP4 rs4986819 (p=0.043). Association with DN were obtained for SNPs: MMP3 rs679620 (p=0.002), ITGB5 rs1007856 (p=0.039), ITGA4 rs1143674 (p=0.028), COL1A1 rs2075555 (p=0.046), ADAMDEC1 rs3765124 (p=0.002), CD247 rs6668182 (p=0.049), PARP4 rs4986819 (p=0.014). Polymorphic variants MMP3 rs679620, ITGB5 rs1007856, ITGA4 rs1143674, ADAMDEC1 rs3765124, PARP4 rs4986819 showed association with both T1D and with DN. Specific for T1D are SNPs of genes LIG1 rs20579, IFNL2 rs12980602, and for DN COL1A1 rs2075555 and CD247rs6668182. Genes MMP3, ITGB5, ITGA4, ADAMDEC1 and COL1A1 involved in the formation of extracellular matrix, collagen metabolism that may lead to fibrotic changes in the renal tubules and interstitium, the progression of DN and the deterioration of the disease. Thus, genes involved into fibrogenesis labeled T1D complication associated with the development of fibrotic processes in the kidney - diabetic nephropathy.

J03.20

Overlapping phenotypes to the Ryanodine Receptor 2 (RyR2) gene

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Type two ryanodine receptor (RyR2) is a Ca2+ release channel on the endoplasmic reticulum (ER) of many type of cells including cardiac and pancreatic b-bells. However the functional role of RyR2 in insulin secretion remains controversial. To determine whether RyR2 channels are involved in glucose homeostasis we took advantage of rare RyR2 mutations that render the

channel "leaky" in patients with a form of exercise-induced sudden cardiac death known as cathecolaminergic polymorphic ventricular tachycardia (CPVT). Here we show that CPVT patients have previously unrecognised profound glucose intolerance. To determine whether leaky RyR2 channels in these patients cause glucose intolerance, we generated mice harbouring CPVT-linked RyR2 mutations. Pancreatic islets from these mice exhibited intracellular Ca2+ leak, activated ER stress response, mitochondrial dysfunction, decreased insulin secretion from pancreatic b-cells and glucose intolerance. Chronic pharmacologic inhibition of intracellular Ca2+ leak improved mitochondrial function and normalised insulin release and glucose intolerance. Thus the RyR2 channels play a heretofore unappreciated role in the regulation of insulin secretion and glucose tolerance.

J03.21

The association of B4GALT1 and IGFBP3 genes polymorphisms with asthma in the Volga-Ural region of Russia.

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Asthma is a heterogeneous disease, usually characterized by chronic airway inflammation. The genetic basis for developing asthma has been extensively investigated. To date, more than thirty GWAS of asthma have been performed and significant associations have been published for nearly 100 asthma genes. The aim of our investigation was to identify asthma susceptibility genes in the Volga-Ural region of Russia. The study included 358 unrelated asthma patients and 369 control subjects of different ethnic origin (Russians, Tatars and Bashkirs). The genotyping was carried out using the Illumina Human610 quad array at the CNG (France) as a part of GABRIEL project. Five markers on chromosome 17q12-21 showed statistically significant association with asthma (p<4,79x10-7). Moreover, we revealed additional two regions of putative association with asthma. The first region was observed at chromosome 9p13 with rs12342831 in B4GALT1 (beta 1,4-galactosyltransferase, polypeptide 1) gene (p=4,232x 10-6); the second region - at chromosome 7p14 with SNP rs1496499 near to IGFBP3 (insulin-like growth factor binding protein 3) gene (p=4,77x10-6). To confirm these associations, we carried out association analysis two SNPs (rs12342831, rs1496499) in independent case-control group (310 asthma patients and 314 control individuals). We detected significant association between two SNPs and asthma in independent group. In summary, this study has revealed and replicated the association between asthma and rs12342831 (9p13), rs1496499 (7p14). Supported by a contract from the European Commission (018996) and the Russian Foundation for Basic Research (13-04-01397).

J03.22

Pseudo-Bartter syndrome: the key to identifying an unknown allele in an infant with cystic fibrosis

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Introduction: Pseudo-Bartter syndrome (PBS) describes a complication of cystic fibrosis (CF) leading to hypochloraemic, hypokalaemic metabolic alkalosis. Aim: To present the case of a seven months baby girl diagnosed with CF at 3 months of age, who presented at 5 months with PBS. Case report: A history of repeated pneumonia, diarrhea and failure to thrive lead to performing a sweat test, which was positive (NaCl 110mM/l - Nanoduct system). We also performed a molecular genetic analysis of the CFTR gene for 29 mutations (kit Elucigene CF 29). It revealed a heterozygous state for F508del-CFTR. The other allele remained unknown. Nutritional therapy and pulmonary therapy was started. The infant was put on an enzyme replacement therapy. Despite the treatment and the family's compliance, her failure to thrive continued. At 5 months she presented with PBS. After the acute phase was resolved, we started supplementing with an oral NaCl solution and the result was an improved growth. The sweat test remained positive after her growth normalised (85mM/l). The kit we used identifies the most common mutations for the Central and Eastern European area. Thus, we performed a wider molecular genetic analysis and the results are pending. Conclusions: The sweat test remains pathognomonic for diagnosing CF. PBS delays the improvement of the nutritional status. Regarding the future pregnancies of the couple, the identification of the second allele is of most importance for offering a proper genetic counseling.

J03.23

Ulcerative colitis is under dual (mitochondrial and nuclear) genetic control

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Introduction: Cellular oxidative stress and genetic susceptibility have been implicated in the multifactorial aetiology of ulcerative colitis (UC). Even though the nuclear genome association with UC has been intensely investigated, the role of the second genome, the mitochondrial DNA (mtDNA), has received far less attention.
Objective: To perform a comprehensive analysis of the mtDNA contribution to UC susceptibility.
Materials and Methods: The association of mitochondrial single nucleotide polymorphisms (mtSNPs) and haplogroups with UC was tested in 488 cases and 833 controls of European ancestry from the NIDDK IBD Genetics Consortium Ulcerative Colitis Genome-Wide Association Study available through dbGaP (study accession number phs000345.v1.p1) and from studies 64 and 65 deposited in the Illumina Genotype Control Database.
Results: No evidence of population stratification could be detected using 218 AIMs for European Americans. 8 mtSNPs were significantly associated with UC but only A10550G in MT-ND4L would survive Bonferroni correction (Punadj=1.40E-07, ORG[95%CI]=4.52[2.46-8.30], 10550G allele: 8.1% of patients and 1.9% of controls). A10550G was equally associated in pairwise conditional analyses with the top GWAS SNPs (7.55E-07<Pcond,unadj<4.42E-06), suggesting that it constitutes an independent risk factor from nuclear-encoded susceptibility loci. Haplogroup K (Punadj=2.76E-02, OR[95%CI]=1.56[1.05-2.32]) and its K1 subcluster (Punadj=2.10E-02, OR[95%CI]=1.65[1.07-2.55]) increase risk for UC while the U5b lineage confers protection against UC (Punadj=2.41E-02, OR[95%CI]=0.40[0.17-0.91]), in accordance to its defining A7768G mtSNP (Punadj=1.50E-02, ORG[95%CI]=0.35[0.14-0.84]).
Conclusion: These results suggest that UC has a dual genetic control (mitochondrial and nuclear) that warrants further replication in independent datasets and reinforces its ethiopathogenic complexity.

J03.24

Utilization of the diagnostic assay Multiplicom CFTR MASTR™ for cystic fibrosis genotyping.

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Cystic fibrosis (CF) screening for population specific panels and large gene rearrangements has improved identification of majority of CF alleles. However, unsolved cases remain making genetic counselling difficult, particularly when the diagnosis is uncertain. Our diagnostic laboratory, involved in cystic fibrosis testing, is currently implementing NGS strategy as method of choice for fast screening of known mutations as well as resequencing of the CFTR gene. The aim of this study was to compare a NGS workflow and Sanger sequencing for specific resequencing of the coding and flanking intron regions of CFTR. We describe a pilot study comprising 8 samples being pre-characterized by Sanger than resequenced using a Multiplicom multiplexing assay for the CFTR gene, and subsequent NGS with MiSeq (Illumina). Data averages in 98% aligned reads, with all exons (except 3 and16) were covered with the mean read depth 1000x. Alternative allele variant frequency was on average 50% and we didn´t detect any mosaic forms of mutations. Within the regions covered by MiSeq-reads, all anticipated variants were detected. In exon 24 we found novel mutation c.4272C>A which has not been detected by Sanger DNA sequencing. Variant calling was performed applying Illumina Variant Studio. Resequencing of the entire coding sequence with NGS platform MiSeq/Illumina increases the sensitivity of mutation detection and is cost effective, especially when samples are multiplexed. Our experience indicates that NGS could be used in routine diagnostics of CF negative for commonly utilised assays.
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J03.25

Different types of mutations are responsible for expression of CAKUT phenotype in Bulgarian population

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Congenital anomalies of kidney and urinary tract (CAKUT) are the leading cause of end stage renal disease in children. They affect 1 in 500 live births and range from renal agenesis, to hypodysplasia, multicystic kidneys, duplex renal collecting system, megaureter, vesicoureteral reflux (VUR), etc. CAKUT may occur in isolation or as a part of syndrome accompanied by non-renal manifestations.

In the present study we performed the largest investigation for molecular pathologies identification in Bulgarian families with CAKUT. A total of 62 patients from 57 families were subjected to genetic testing. In the non-syndromic cases, TCF2, PAX2, SALL1, EYA1 and SIX1 genes were screened for mutations by Sanger sequencing and MLPA. In the CAKUT cases with complex pathological phenotype an array Comparative Genomic Hybridization (aCGH) was applied for detection of different chromosomal defects.

Various novel and known pathogenic mutations were found in a number of families such as missense variants and insertions in TCF2 and SALL1, partial or whole gene deletions affecting TCF2 and LPP, as well as chromosomal aberrations involving the 6p25 locus, the CFHR gene cluster and the entire chromosome 8. These defects explain the pathology in 11.5% of the families. Additional analysis are required for elucidating the genetic cause of CAKUT in the remaining cases.

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J04.01

FBN1 gene analysis in suspected Marfan syndrome patients using a combined molecular genetic workflow

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Marfan syndrome is an autosomal dominant connective tissue disorder. Early establishment of the diagnosis is important because of severe cardiovascular complications of the disease. Sequencing of the fibrillin-1 (FBN1) gene has a 70-90% clinical sensitivity in patients with Marfan syndrome. We developed a rapid and reliable diagnostic workflow for FBN1 gene analysis by combining Sanger and next generation sequencing (NGS) methods.

Exons and flanking regions of the FBN1 gene were amplified in 65 amplicons. Homopolymer-containing exons (n=16) were sequenced using Sanger technology while all other amplicons were sequenced using NGS (Roche GS Junior), where coverage criteria was above 40x. Pathogenic mutations detected by NGS method were confirmed by Sanger sequencing.

Twenty-three families were tested. Marfan syndrome was confirmed in 10/23 unrelated patients. Eight missense and two small scale deletion/duplication mutations were detected among which six were novel alterations (c.5196delC, c.2585G>A, c.6032G>C, c.3038G>T, c.2288G>A and c.2272_2275dupTCAA). Combining NGS and Sanger sequencing methods we could establish reliable FBN1 gene analysis for Marfan syndrome diagnostics.

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J04.02

Worldwide recurrent missense mutation of the PTPN11 gene in a Hungarian patient with LEOPARD syndrome

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LEOPARD syndrome (LS, OMIM 151100) is a rare monogenic disease. Its name is an acronym for the major features of this disease including multiple lentigenes, electrocardiographic conduction defects, ocular hypertelorism, pulmonary stenosis, genital abnormalities, growth retardation and sensorineural deafness. LS develops due to mutations in the protein-tyrosine phosphatase nonreceptor-type 11 (PTPN11) gene. Recently, we have identified a 51-year-old Hungarian man with LS. Direct sequencing of the PTPN11 gene revealed a worldwide recurrent missense mutation (c.836A/G; p.Tyr279Cys) in the PTPN11 gene. The detailed description of the Hungarian patient and the review of the previous studies reporting the same mutation are presented in this study. Elucidation of the genotype-phenotype correlations may promote the understanding of the mechanism and may contribute to the development of future therapeutic modalities.

J04.03

Novel mutations in two Hungarian sisters with oculocutaneous albinism type IV and reviewing the literature

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Oculocutaneous albinism is a group of rare monogenic diseases characterized by decreased or absent pigmentation in the hair, skin and eyes. Due to the abnormalities of the pigmentation, the affected patient may suffer from photosensitivity and develop cancers of the skin. In addition to the variety clinical symptoms, the genetic background of this group of diseases is also heterogenic. In this study, two Hungarian sisters affected by oculocutaneous albinism were investigated. The clinical symptoms of the patients suggested the presence of the OCA type 2 or type 4 variant, therefore direct sequencing of the P and SLC45A2 genes were performed. Our results revealed two novel heterozygous mutations in the SLC45A2 gene: a missense mutation in exon 6 (c.1226G/A, p.Gly411Asp) and a nonsense mutation in exon 7 (c.1459C/T, p.Gln437X). Both patients are in compound heterozygous state and carry both mutations. The detailed description of the Hungarian patients and the review of the previously published mutations are presented in this study. Elucidation of the genotype-phenotype correlations may promote the understanding of the mechanism and may contribute to the development of future therapeutic modalities.

J04.04

Study of FBLN5 and LOXL1 genetic variations in association with pelvic organ prolapse taking into account levator trauma and parity

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Pelvic organ prolapse (POP) is a common urogynecological disease with a strong hereditary component. Traumatic and multiple childbirths may cause damage of pelvic ligaments and overdistention of the vagina and thereby increase the risk of POP developing. The proteins fibulin-5 (FBLN5) and lysyloxidase 1 (LOXL1) involved in elastic fiber synthesis and connection play an important role in POP development.

We analyzed the association of polymorphic variants in the genes of *FBLN5* and *LOXL1* with POP taking into consideration traumatic childbirths and parity. Groups under study included 210 females with POP and 291 matched controls. HaploView resource was used to select a set of "tag" SNPs. Nine *FBLN5* SNPs and three *LOXL1* SNPs were genotyped using the PCR reaction with confronting two-pair primers. After the stratification of the sample, we found the association of some alleles of *FBLN5* and *LOXL1* genes with POP in the groups with perineal trauma and ≥ 2 childbirths (Table 1). These data may be used to identify risk groups for POP development.

This work was supported by RFBR (grant 15-04-02378).

Table 1. Association of polymorphic variations in the *FBLN5* and *LOXL1*genes with pelvic organ prolapse in the groups with traumatic and multiple childbirths

tagSNPs	Perineal trauma			≥ 2 childbirths		
	Control	POP	*P*–value, OR,95%CI	Control	POP	*P*–value, OR,95%CI
	Number			Number		
rs2018736-C *FBLN5*	n=86	n=104	0.0033(dom) 2.52(1.35-4.71)	n=98	n=118	0.0084(ad) 1.71(1.14-2.57)
rs12589592-A *FBLN5*	n=92	n=105	0.0018(rec) 0.27(0.11-0.64)	n=106	n=118	0.0081(rec) 0.38(0.18-0.79)
rs2474028-T *FBLN5*	n=89	n=105	0.028(dom) 1.96(1.07-3.59)	n=101	n=117	0.04(dom) 1.81(1.02-3.22)
rs12586948-A *FBLN5*	n=91	n=105	0.047(dom) 1.83(1.00-3.35)			
rs2304719-T *LOXL1*				n=105	n=115	0.045(dom) 1.77(1.01-3.09)

J04.05

A search for genetic susceptibility marker to osteoarthritis in women with connective tissue dysplasia

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The study of polymorphisms rs2228570 (s.2T/A/C/G, Met1Lys/Arg/Thr; FokI, rs10735810), rs1544410 (c.1024+283G>A; BsmI), rs7975232 (c.1025-49G>T, ApaI), rs731236 (c.1056T>C, TaqI) receptor gene of vitamin

D (VDR), was held in women suffering from osteoarthritis (OA) and showing signs of connective tissue dysplasia (CTD). The material for the study were DNA samples of 255 women aged from 23 to 61 years, mean age 51,4 ± 2,2 years. OA was diagnosed according to the criteria of the American Association of Rheumatology (1995). Availability CTD evaluated clinically using phenotypic table by TI Kadurina.
Significant influence of polymorphisms rs1544410 (c.1024+283G>A; BsmI) and rs7975232 (c.1025-49G>T, ApaI) VDR gene in the development of both OA and DST alone or with a combination of these pathological conditions was found. The largest contribution to the increase in the risk of developing OA and DST makes allele *G and genotype *G*G of BsmI locus. Genotype *A*A BsmI locus is a marker of reduced risk of CTD. Genotype *G*T ApaI locus increases risk of OA with CTD. Genotype *T*T locus ApaI is a marker of reduced risk of CTD as a whole, as well as the development of comorbidity. *CGG haplotype BsmI, ApaI, TaqI loci associated with an increased risk of connective tissue dysplasia, haplotype *CTA reduces the risk of comorbidity.

J04.06
Novel RUNX2 splicing mutation in a patient with cleidocranial dysplasia

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Introduction: Cleidocranial dysplasia (CCD) is a rare autosomal dominant disorder caused by haploinsufficiency of Runt-related transcription factor RUNX2, which affects osteoblast function during endesmal osssification (and other processes), leading to clavicle hypo(a)plasia, persistent skull sutures, dental anomalies and other problems. In a patient with clinical diagnosis of CCD, we searched for the causal mutation in RUNX2.
Materials and Methods: All 9 exons of RUNX2 (longest isoform, NM_001024630.3) were amplified by PCR from genomic DNA isolated from peripheral blood and subjected to Sanger sequencing. For splicing detection, peripheral blood RNA was reversely transcribed and amplified with primers spanning exons 4-6.
Results: Patient is a 42 year old female diagnosed during childhood with CCD for bilateral aplasia of clavicles, impaired skull ossification, persistence of deciduous teeth, flatfoot and hip dysplasia. Sequencing of genomic DNA revealed G->A substitution at postition +5 in intron 5, not listed in dbSNP. Splicing prediction by three different tools showed modest drop in score, so we tested actual splicing by RT-PCR. Exon 5 was skipped in about 30% of transcripts, regarded to be enough to cause pathology. Interestingly, skipping exon 5 (105bp) does not lead to a frameshift. The resulting protein lacking part of the Runt domain and a nuclear localization signal may be therefore translated and may potentially represent a dominant negative.
Conclusion: In a patient with typical cleidocranial syndrome manifestation, a novel splicing variant was found in intron 5 donor splice site.
Supported by Charles University in Prague, Czech Republic (PRVOUK P24/LF1/3).

J04.07
Rare case of skeletal dysplasia - multicentric carpotarsal osteolysis (MCTO) caused by MAFB gene mutation.

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Multicentric Carpotarsal Osteolysis (MCTO) is a rare type of skeletal dysplasia characterized by progressive osteolysis, affecting the carpal and tarsal bones and is frequently associated with renal failure (OMIM 166300). MCTO is monogenetic autosomal dominant disease caused by MAFB gene mutation.
We report 10-years old boy with MCTO diagnosed by sequencing of MAFB gene. Patient was born in the proper delivery time in a good condition, no developmental abnormality was noticed. When the boy was 2 years old he started to limp. When he was 4 he suffered while walking, his ankles and wrists were swollen. Juvenile rheumatoid arthritis was suspected. After some time, he could walk only on his heels. Besides the chronic renal failure was diagnosed. Moreover, intellectual disability type border-line is observed. In the tests of bones symptoms of osteoporosis and osteopenia and destruction of metaphyses structure was diagnosed. Dysmorphic facial features such as: small face, a slim nose, long eyelashes, maxillary hypoplasia, small mouth and thorax dysmorphism (slopping shoulders, narrow and carinatum thorax) and muscle weakness and atrophy of upper and lower extremities were also observed.
After clinical diagnosis of MCTO, MAFB gene mutation screening was performed by direct sequencing. Pathogenic de novo mutation (Pro59Leu) was revealed.
MCTO is progressive disease affecting skeletal system and kidneys. MCTO is usually presenting in early childhood with a clinical picture mimicking juvenile rheumatoid arthritis. Differential diagnosis of patients with coexistence of joints and renal problems should include MCTO as one of possible cause.

J04.08
Ectrodactyly and Ectodermal Dysplasia without cleft lip/plate: a case report

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Ectrodactyly or Split hand/foot malformation (SHFM), may occur either as an isolated malformation or coexists with other congenital anomalies (cleft lip/palate, ectodermal dysplasia or developmental delay/mental retardation). There are several relatively frequent syndromes in which ectrodactyly occurs as one of the multiple congenital anomalies. The most common involve EEC syndrome (Ectrodactyly, Ectodermal dysplasia, Cleft lip/palate syndrome) and EEC syndrome without cleft lip/palate.
We describe this rare disorder in a boy, who had ectrodactyly and ectodermal dysplasia, without cleft lips/palate. He is the only child of healthy nonconsanguineous parents. Pregnancy was complicated by threatened miscarriage and oligohydramnios and he was born full term. Birth weight was 2.300 kg. At birth he showed multiple morphologic abnormalities: ectrodactyly with agenesis of the third and forth ray and metacarpus in the right hand, agenesis of the third ray in the left hand, soft tissue syndactyly and median cleft in both hands, nail alterations to the toes, right preauricular appendix, sparse and thin hairs and eyebrows, xerosis with diffuse lamellar desquamation at the back, perineal hyperemia with disepithelization areas, agenesis of left kidney, cryptorchidism, micropenis, ostium secundum atrial septal defect. At 7 months of age his weight was 5.850 kg (<5°P), length 61.5 cm (<5°P) and presented psychomotor delay. Hair analysis showed: "Trichorrhexis nodosa, Pili trianguli et canaliculi, Trichoschisis". Karyotype analysis, Array-CGH and molecular analysis of TP63 gene were normal. We planned to perform molecular analysis of other genes involved, such as WNT10B and DLX5, to identify the molecular basis of this form of SHFM.

J04.09
Molecular characterization of the natural occurring SERPINH1 Dachshund model of Osteogenesis imperfecta

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Osteogenesis imperfecta (OI) is a heritable connective tissue disease characterized by increased bone fragility, fractures and deformities of the skeleton. Mutations in at least sixteen genes have been associated with dominant and recessive forms of OI. SERPINH1 encoding the ER collagen chaperone HSP47 was identified to cause a severe form of OI in Dachshunds (Leu326Pro) and in one human patient.
To elucidate the disease mechanism underlying OI in the dog model, we applied a range of biochemical assays to mutant and control skin fibroblasts as well as on bone samples.
Type I collagen synthesized by mutant cells had decreased electrophoretic mobility. Procollagen was retained intracellularly with concomitant dilation of ER cisternae and activation of the ER stress response marker GRP78. In line with the migration shift detected on SDS-PAGE of cell culture collagen, extracts of bone collagen from the OI-dog showed a similar mobility shift and on tandem mass spectrometry the chains were post-translationally overmodified. The bone collagen had a higher content of pyridinoline than control dog bone.
The SERPINH1 mutation in this naturally occurring model of OI impairs how HSP47 acts as a chaperone in the ER. This results in abnormal post-translational modification and cross-linking of the bone collagen.

J04.10

Testing the association between rs689, rs680, rs3767140, rs1800469 polymorphisms and the 2d:4d ratio in diabetic patients

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Introduction: The 2d:4d ratio was considered a multifactorial trait, showing sexual dimorphism. In men the 2nd finger is shorter than the 4th finger, whereas in women the two fingers have approximately the same length. Insulin, IGF2, TGFb and HSPG gene products are involved in body growth, development and shaping, mainly during fetal development. Patients with diabetes present altered microcirculation and impaired matrix and bone mineral density. The aim of this study was to test the association between rs689, rs680, rs3767140, rs1800469 polymorphisms and the2d:4d ratio in patients with T1DM, T2DM and control subjects.
Materials and Methods: The study involved 320 individuals distributed into 4 groups: T1DM (80 patients with T1DM; 25,9 ± 4,1 years), HC1 (80 clinically and paraclinically healthy subjects, 26.4 ± 3.9 years),T2DM (80 patients with T2DM; 54,7 ± 3,3 years), HC2 (80 clinically and paraclinically healthy subjects, 53.6 ± 3.4 years). The two sexes were similarly distributed within the groups. Finger length was measured using digital callipers. Blood from each subject was collected and used for DNA extraction (Wizard®Genomic DNA Purification Kit), that was later used for polymorphisms genotyping, by PCR-based methods.
Results: The AA genotype of the rs689 polymorphism was strongly associated with T1DM (AA OR = 4.26; p <0.0001). The 2d:4d ratio was associated only with gender in T1DM and HC1 lots (p= 0.0001). This ratio was associated only with rs680 în T2DM and HC2 lots (p= 0.001).
Conclusions: The 2d:4d ratio was significantly associated with gender in T1DM patients and control subjects, and with IGF2 Apa polymorphism in T2DM patients.

J04.11

A Recessive Skeletal Dysplasia, Novel Type, Results from a Mutation of NEK1 (Never in Mitosis Gene A-Related Kinase 1)

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Introduction: This study is the analysis of a nuclear consanguineal family with two affected (13 year old girl and a 7 year old boy) and one 3 month old boy with similar clinical features identified an autosomal-recessive form of skeletal dysplasia. There were no other cardiological and abdominal assessment findings. On the basis of the clinical features and laboratory findings, we performed whole-exome sequencing for one subject.
Materials and Methods: Two affected children were characterized by dysmorphological findings as narrow forehead, hypertelorism, pectus excavatum, prominent eyes. There were unique constellation of radiographic findings including biconcave vertebrae, coxa vara, enlarged humerus diaphysis and femur metaphysis in both affected children that the boy had more severe features. Chromosomal analysis revealed normal karyotypes. Furthermore, we performed whole-exome sequencing for one subject and identified a novel mutation (c.3630+1G>C and c.G3498T:p.K1166N) in the NEK1 (Never in Mitosis Gene A-Related Kinase 1, OMIM *604588) gene. We have confirmed this homozygous mutation in other affected children; where as, the heterozygous mutation cosegregated with other family members.
Conclusion: NEK1 (Never in Mitosis Gene A-Related Kinase 1) gene is located on 4q33 that encodes a protein kinase and its‘ defects cause short rib-polydactyly syndrome type 2. According to the findings, we have suggested that this is a new type skeletal dysplasia, indicating autosomal recessive inheritance.

J04.12

Case presentation: A one year old girl with the diagnosis of Cutis Laxa syndrome

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• A one year old girl with respiratory distress, tachypnea, cough, fever, coarse crackles, hypotonia and developmental delay. She was the consequence of first cousins marriage. She had a healthy older brother. Her clinical manifestations were: H.C = 41.5 cm, length= 69 cm, weight: 6200, deep set eyes, brachycephaly, mild mid face hypoplasia, micrognathia, long eyelashes, frontal bossing, hyperextensible joints (joint hyperlaxity), flexion deformities in hands (fingers) and wrists, hypotonia, transverse palmar crease, umbilical hernia, cranial sutures overriding, soft and doughy skin, cutis laxa, easy bruising, mongolian spot (right flank), short neck, wide nipples. Her paraclinical findings were: normal echocardiography, normal brain MRI, normal metabolic screening.
The differential diagnoses for this patient were:
1. Ehler Danlos Syndrome (Dermatosparaxis type, AR)
2. The Collagen related Myopathies such as Ullrich Congenital Muscular Dystrophy (AR)
3. Inherited Cutis Laxa (AR)
The result of molecular study for the most probable diagnosis (Cutis Laxa) was positive and she was homozygote for mutation in PYCR1 gene.

J04.13

Two DMP1 synonymous mutations in a Malaysian hypophosphatemic rickets patient

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Hypophosphatemic rickets (HR) is a rare disorder of bone mineralization due to defective phosphate reabsorption in the renal tubules. *PHEX*, *FGF23* and *DMP1* are the associated genes of familial HR that contribute to X-linked dominant, autosomal dominant and autosomal recessive HR, respectively. This study is aimed to identify the underlying genetic mutation in a four-year-old girl who manifested clinical features of HR and affirmed by biochemical tests and radiological analysis. Her parents did not show any clinical symptoms of HR. Mutational analyses of *PHEX*, *FGF23* and *DMP1* were carried out by polymerase chain reaction and direct sequencing of coding exons and flanking intronic regions. No mutations were found in *PHEX* and *FGF23*. However, synonymous mutations were found in the patient at two different locations of exon 6 in *DMP1*; c.1322C>T, and c.1334G>A. These mutations did not change the amino acids; S406S and E410E, respectively. RNAfold, a software that predicts the secondary structure of RNA, foretells that these synonymous mutations affect the secondary structure of mRNA, resulting in lower stability and translation efficiency. The presence of two synonymous mutations in exon 6 of *DMP1* is possibly the cause of this disorder in the child. Genetic testing for HR patients is necessary for correct early diagnosis, treatment intervention, counseling and to help improve patient clinical care and management.

J04.14

The study of *IL1A* gene polymorphisms as risk factors for Psoriatic Arthritis in Romanian population

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Introduction. Psoriatic arthritis (PsA) is a chronic disorder characterized by skin psoriasis and the inflammation of the peripheral joints and/or spine. PsA belongs to the group of spondyloarthritides and shows strong association with SNPs in the major histocompatibility complex region, but genes outside this region have also been implicated in disease pathogenesis. Interleukin 1 alpha (IL-1α) is a proinflammatory cytokine involved in the regulation of immune responses and inflammatory processes.
Genetic variations of IL1 locus have been associated with the risk of spondyloarthritides.Our aim was to investigate two IL1A gene SNPs for association with PsA.Materials and methods. The study included 97 PsA patients and 160 healthy unrelated controls, all of Romanian origin. The two SNPs of IL1A gene rs1800587 (-889C>T) and rs17561 (Ser114Ala) were genotyped by TaqMan Allelic Discrimination Assay (7300 Real time PCR System, Applied Biosystems, USA). Alleles, genotypes and haplotypes frequencies were compared using the software PLINK v 1.07 and p values ≤0.05 were considered significant. Results. There was no statistically significant difference in allelic, genotypic or haplotypic frequency distribution between cases and controls. We also tested whether the investigated SNPs were associated with clinical features, but no significant differences were detected. Conclusions. Our results suggest that the two IL1A gene markers do not influence the risk of PsA

in Romanian population. However, the number of individuals analysed could be insufficient to evaluate small genetic effects. Additional studies with enlarged sample size are needed to clarify the role of IL1A polymorphisms in PsA.Grant support: SOP HRD/159/1.5/S/135760

J04.15
Pycnodysostosis : Report of three Tunisian cases

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Pycnodysostosis is an autosomal recessive lysosomal disorder due to CTSK gene (1q21) mutations. This rare skeletal dysplasia is characterized by short stature, acroosteolysis of distal phalanges and frequent fractures due to bone fragility. Cranio-facial dysmorphy including a delayed suture closure with open anterior fontanelle, an exophthalmia, a prominent nose, an obtuse angle mandible and dental anomalies is commonly noted.
In this report, we describe the clinical and radiological features of three Tunisian patients (one familial and one sporadic case) with pycnodysostosis.
These patients were referred to the authors' department for evaluation of a short stature and a facial dysmorphy.
All were girls, born from consanguineous parents and their ages ranged from 9 to 12 years. The eldest of two sisters had, at the age of 9, a fracture of the right tibia which occurred after a trivial injury. All of our patients had normal psychomotor development and were normally schooled.
The clinical examination revealed a height between -4 and -5 SD, short and stubby fingers, and dysmorphic features including frontal bossing (3/3), open anterior fontanelle (3/3), exophtalmia (3/3), a prominent nose (3/3), hypoplasia of the mandible (3/3), malapposed teeth (2/3) and narrow palate (1/3).
Skull and hands radiographies showed open sutures and acroosteolysis of terminal phalanges, respectively.
These clinical and radiologic features enabled us to retain the diagnosis of pycnodysostosis and consequently to offer an appropriate genetic counseling and an adequate orthopedic and stomatologic follow up. However, CTSK gene analysis should also be realized in order to determine the molecular profile of Tunisian patients with pycnodysostosis.

J04.16
Polymorphism rs 3134069 OPG gene in postmenopausal women with osteoporosis

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Introduction: Osteoporosis is a common disease that is characterized by low bone mineral density (BMD), deterioration in bone microarchitecture, and increased fracture risk.
Materials and Methods: The aim of this study was to find distribution of genotypes of polymorphisms T245G (rs3134069) OPG gen in control group of postmenopausal women (n=104) and in osteoporotic group of postmenopausal women (n=105). Genomic DNA was isolated from leucocytes of peripheral blood using standard methods. Genotyping was realized by TaqMan SNP genotyping assay (Applied Biossystem) on basis of standard protocol. Fluorescence was detected by Real-Time PCR method using apparatus StepOne™ Real-Time PCR System.
Results: Distribution of surveyed genotypes in osteoporotic group was: TT (80,00%), TG (20,00%), GG (0,00%). Distribution of genotypes in control group was: TT (85,58%), TG (13,46%), GG (0,96%). Statistical significance between behalf of genotypes of control and osteoporotic group was not found.
Conclusion: Molecular-genetic research represents the best way in order to create complex view over genetic conditionality of osteoporosis.
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J04.17
Autosomal recessive congenital ichthyoses in the Czech Republic

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Introduction: Autosomal recessive congenital ichthyosis (ARCI) is a heterogeneous group of disorders of epidermal cornification caused by mutations in one of at least nine genes. Methods: ARCI families presenting at our laboratory over a period of 3 years were assessed using PCR-direct sequencing and/or Sequence Capture and Targeted Resequencing. The genetic results were complemented by analysis of missense mutations using homology modelling on the basis of known 3D protein structures.
Results: The genetic analyses were performed in 34 Czech ARCI probands: 11 patients carry mutations in ALOX12B, 6 patients in ALOXE3, 6 patients in NIPAL4, 4 patients in CYP4F22, 3 patients in TGM1, 1 patient in ABCA12, and 3 patients are without causal mutations despite that all known genes up to now associated with ARCI were analysed. Totally, mutations were identified in 31 patients (91.2%). In silico analysis of missense mutations in ALOX12B, ALOXE3, and TGM1 genes revealed various structural defects while no defects were associated with known polymorphisms.
Conclusions: Besides identification of the spectra of mutations in Czech EB patients, the study present new method implemented into genetic diagnostics of genodermatoses in the Czech Republic - sequence capture and targeted resequencing - that provides more complete diagnosis than a classical gene-by-gene approach. Parallel analysis of known genes in a patient (or in multiple patients) enables fast and cost-effective identification of gene mutations.
Currently, we are able to perform analysis of 81 genes associated with different inherited skin disorders.
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J04.18
Molecular genetic analysis of Fibrodysplasia ossificans progressiva in Russian patients.

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Fibrodysplasia ossificans progressiva (FOP, MIM 135100) is a rare disease with autosomal dominant inheritance, characterized by genetically determined ossification of the connective tissue of muscles, fascia and tendons different localization. FOP different progressive course, leads to significant violations of the functional state of the musculoskeletal system, profound disability of patients, up to complete immobilization. Attempts to surgical removal of ossification cause new recurrence of the pathological process.
ACVR1 mutations responsible for the development of FOP. This gene localized in the locus 2q23-q24. In the laboratory our Center is searched ACVR1 gene mutations in patients with FOP by direct analysis of DNA sequencing of all exons and exon-intron junctions. 37 patients were examined. Found 13 mutations. In 11 cases revealed the most common mutation c.617G>A (p.Arg206His). In two other cases found mutations c.619C> G (p.Gln207Glu) in exon 7 and c.1067G>A (p.Gly356Asp) in exon 10 of the gene ACVR1. The investigations of this gene are in progress now.

J04.19
Unbalanced translocation associated with acrania in two subsequent pregnancies

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Objectives: The aetiology of NTDs is heterogeneous.
Method: Ultrasound evaluation, karyotype analysis, SNP array (Illumina), IVF with PGD, IUI with donor sperm
Results: 25-year-old primigravida in 2010 with high level of MSAFP and ultrasound finding of acrania in the 16th week of pregnancy. Amniocentesis was performed with abnormal karyotype 46,XY,add(1)(p 36). Subsequent parental karyotype analysis revealed paternal balanced translocation 46,XY,t(1;3)(p36.3.;p24.2) de novo. In 2011 the second spontaneous pregnancy was checked in the 12th week by ultrasound and a fetus with acrania was detected again. We went on with SNP array analysis. The result was the deletion of 3Mb in the region 1p36.33- p36.32 and the duplication of 22.6Mb in the region 3p26.3-p24.3. It meant an involvement of great amount of genes with different functions. Then in 2012 the couple decided to undergo IVF with PGD. Based on this treatment, 5 embryos were created, all were unbalanced. The subsequent pregnancy in 2012/2013 was performed by IUI with a donor sperm, without any invasive investigation, which led to a successful birth of a healthy boy.
Conclusions: Despite the implementation of new genetic technologies, our family delivered a healthy child only due to a donor sperm. A key role for the detection of a fetus with acrania is the first trimester ultrasound examinati-

on. Later detection includes a high level of MSAFP in the second trimester.
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J04.20
N- terminal region of human low-molecular weight salivary mucin gene (MUC7) N80K polymorphism may be a biomarker for dental caries.

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Purpose: Human low- molecular- weight salivary mucin (MUC7), also known as MG2, is a small, secreted glycoprotein that functions to help salivary defence against oral bacteria by masking their surface adhesins and thereby inhibiting colonization. In this study, we aimed to identify the N- Terminal region of MUC7 gene polymorphisms between individuals with and without caries.
Methods: 44 Healthy dental students were included in this study. 24 of them were classified to have dental caries (DMF-T=5,6) according to the World Health Organisation (WHO) and 20 of them were caries- free (DMF-T=0). Salivary total protein was measured by Lowry method, salivary buffer capacity (SBF) by Ericsson's method. Saliva flow rate and pH was also calculated. Genotyping procedure was carried out by PCR- direct sequencing.
Results: We detected a transversition of C to G, which causes a change asparagine to lysine in codon 80 (N80K). This alteration was found 33,3% and 42,1% in with and without caries groups, respectively. There was no statistically significant difference between caries and non- caries group in the terms of biochemical and genetic results.
Conclusion: The SNP found in this study may be a specific polymorphism affecting Turkish population. Further studies with more numbers of individuals are necessary in order to clarify this finding.

J04.21
Interdisciplinary management of a young patient with hypodontia :a orthodontic-restorative-surgical approach

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Hypodontia represents the congenital lack of one or a few teeth and is an autosomal inherited dominant trait. Although a lot of progress has been made in understanding the causes of hypodontia, knowledge of the exact aethiology remains poor. Genetic studies discovered mutations in 3 genes in patients with familial hypodontia or oligodontia: MSX1, PAX9 and AXIN2.
The patient, a 22 years old male was referred to the Department of Orthodontics, University of Dentistry Tîrgu-Mures, with a chief complaint of missing permanent teeth. Extraoral examination revealed a well-balanced face with normal facial profile and normal skeletal dental base relations. Intraoral examination revealed a mixed dentition. Radiographic examination confirmed that the following teeth were developmentally missing: 4.2,3.3,4.1,1.2,1.5.
A multidisciplinary team involving an orthodontist and a prosthodontists established the treatment plan and the clinical management of this case. A combined orthodontic-restorative-surgical approach was adopted. The aim of the orthodontic treatment was dental allignement and creation of space for placement of implants. Orthodontic treatment was conducted using a fixed staight-wire appliance for 12 months. Implants were placed and after five months the final prosthetic restauration was performed.
Young patients with congenitally missing teeth need an early refferal to a dentistry team for optimal management. This case report shows the need for a multidisciplinary team approach for the establishment of the treatment plan. The two main objectives for patients with hypodontia, namely improved esthetics and restoration of masticatory function, were achieved in this case.

J04.22
Deregulated expression of genes involved in extracellular matrix regulation in tendons of patients with rotator cuff tear

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Rotator cuff tear is one of the most common shoulder dysfunction. Gene expression analysis is a useful tool for understanding tendon tears. TGFβ1 is a cytokine that seems to be involved in tendon differentiation. TGFβR1 is one of the main receptor of the TGFβ pathway. GDF5 and KFL6 are members of the TGFβ family. Moreover, FN1 and TNC glycoproteins seems to have a role in the tendon healing. No previous study evaluated the mRNA expression of these genes in tendons of patients with rotator cuff tears. We evaluated TGFβ1, TGFβR1, GDF5, KFL6, FN1 and TNC expression in 28 ruptured supraspinatus tendon samples of patients with rotator cuff tears and in 8 normal supraspinatus tendon samples by reverse-transcription quantitative polymerase chain reaction. HPRT1, TBP and ACTB were selected as reference genes for gene expression normalization. We observed that FN1, TNC and TGFβR1 expression was increased in tendon samples of cases compared with controls (p=0.002, p=0.025, p=0.020, respectively). Increased FN1 (p=0.025) and TNC (p=0.014) expression was detected in smokers in relation to non-smoker patients. Samples with microcyst presented increased TNC expression in relation to samples without microcysts (p=0.046). Samples with microcysts also tented to present higher FN1 expression. (p=0.054). Samples with myxoid alterations presented reduced GDF5 expression (p=0.029). In conclusion, FN1, TNC and TGFβR1 expression were deregulated in tendons of patients with rotator cuff tears. These molecular alterations may lead to modifications of tissue structure and of the healing process, as well as to tendon ruptures.
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J04.23
FOXL2 mutations in Iranian patients with blepharophimosis-ptosis-epicanthus inversus syndrome

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Blepharophimosis-ptosis-epicanthus syndrome (BPES) is a rare genetic disorder with autosomal dominant inheritance. There are two distinct phenotypes: BPES type I, which is associated with female infertility or premature menopause due to ovarian resistance to gonadotropins, whereas in type II only eyelid abnormalities are present. Mutations in the forkhead transcription factor 2 (FOXL2) gene are responsible for both types of BPES. The purpose of this study was to identify mutations in FOXL2 in Iranian patients with BPES.
The peripheral blood was collected from the patients of two families and genomic DNA was extracted. Direct sequencing of all exons of FOXL2 gene was performed.
Two mutations in FOXL2 were identified in two familial cases c.102-103 ins A (p.G35R fsx), a novel mutation, and c.855-871 dup (17-bp insertion), a known disease-causing mutation were found in FOXL2 gene. These mutations cause truncated protein and envisaged to have severe phenotypic impact in harmony with type I phenotype seen in the patients.
The role of FOXL2 gene in the etiology of infertility is still unclear, but several studies suggested that it plays a central role in follicle development. Our results expand the spectrum of FOXL2 mutations and confirm the mutations association with eyelid abnormalities and female infertility.
Acknowledgments:
This work was supported by a grant from Tehran Medical Genetics Laborator

J04.24
Cathepsin K mutation is responsible for pycnodysostosis in a Turkish family

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Introduction: Pycnodysostosis (PKND) is a rare, autosomal recessive disease characterized by short stature, severe bone fragility, exophthalmus and oral manifestations such as micrognathia. It usually demonstrates typical craniofacial deformities, such as hypoplastic midface, anterior cross-bite, grooved palate and dental crowding. The aim of this case report is to investigate the role of cathepsin K (CTSK) for pycnodysostosis in an effected Turkish family.
Material- Method: Family applied to Marmara University, Department of Pedodontology for treatment of PKND. Three of five siblings had PKND whereas the other two siblings were PKND-unaffected. All the siblings' detailed clinical and radiological examinations were performed.
Results: Taking into consideration the fragility in these patients, appropriate restorative treatments and protective applications were preferred. Patients were given oral hygiene education. Recall visits occurred in every 3 months during the 18-month follow-up period. For mutational analysis, genomic DNA was isolated from buccal cells. Seven exons, including exon- intron

boundaries of CTSK were directly sequenced after amplification in all family members.
Conclusions: Early diagnosis and treatment is important with PKND patients, since bone fractures easily occur during dental treatment and limited mouth opening makes it difficult to access the treated area. Frequent recalls should be planned for PKND patients. Regular oral care and early preventive treatments ensure the patients a better life quality. Genetically, all the affected members carried L7P variation as homozygous state, whereas unaffected had the heterozygous state of the same condition. Conclusively, we suggest that L7P mutation is important for the onset of this anomaly.

J04.25
Familial recurrence of lethal neonatal coagulopathy: Novel mutation in arysulfatase-E suggests severe phenotype of x-linked chondrodysplasia punctata

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Two male babies were born to identical twin mothers and both mothers were completely deficient in vitamin K. Both patients were born in extremely poor condition with disseminated intravascular coagulopathy (DIC) and dysmorphic features; small noses with depressed nasal bridges and midface hypoplasia. Radiographs revealed stippled epiphyses and tracheal ring calcification. Both infants died in the neonatal period from haemorrhagic sequelae.
Targeted sequencing of X chromosome exons revealed both patients to be hemizygous for a novel mutation (c.1692C>G) in exon 11 of arysulfatase-E (*ARSE*), encoding the Golgi enzyme arylsulfatase-E. The mutation was carried by both mothers and was demonstrated to have arisen *de novo*. The mutation is predicted to destabilise the ARSE protein and reduce overall protein activity.
X-linked recessive chondrodysplasia punctata (CDPX1) refers specifically to mutations in *ARSE* leading to the clinical features of distal phalangeal hypoplasia, stippled epiphyses and typical dysmorphic facial features (Binder face). Mutations in *ARSE* are found in 50% of patients with CDP, suggesting that in the remaining patients, clinical features are caused by mutations in other genes or, more commonly, by non-genetic phenocopies.
Our data supports the pathogenicity of this novel mutation and clinical features are consistent with a lethal phenotype of CDPX1. Three further cases are reported in the literature of neonatal haemorrhage associated with mutations in *ARSE*; this suggests that a severe sub-group of CDPX1 exists. The substrate for ARSE is currently unknown but our patients provide more evidence for the role of vitamin K in this pathway and potential therapeutic targets.

J04.26
Missing heritability in Primary Osteoarthritis: the EXORHUM project

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Osteoarthritis (OA) is the most common joint disease observed worldwide and affects nearly 10 million adults in France and 27 million adults in USA. OA is mainly characterized by the gradual loss of the articular cartilage, particularly affecting the knee, hip, spine, hand and foot. Although the multifactorial nature of the vast majority of OA cases is well recognized, genetic risk factors have been found to be strong determinants of the disease. About 50% of hip and hand OA may be genetically determined. However, currently, only few genetic alterations responsible for OA determinism have been identified mostly in genes involved in the cartilage extracellular matrix. These alterations have been mainly detected in patients with syndromic early-onset OA like pseudoachondroplasia, multiple epiphyseal dysplasia or spondyloepiphyseal dysplasia tarda.
The EXORHUM project is based on the recruitment of a well clinically characterized cohort of patients with non syndromic early-onset OA. The main aim is to identify new genetic alterations, exclusive to primary OA in patients with a familial presentation, strongly suggestive of a genetic determinism. Exome sequencing will be performed on two distant affected family members without known alterations. The sub aim is to assess the prevalence of known mutations in primary non syndromic OA.
In conclusion, the EXORHUM project will help us to improve the understanding of molecular processes involved in pathogenesis of OA and to better understand the complexity of OA. This is essential to improve patient diagnosis and to identify new therapeutic targets.

J04.27
Premature ovarian failure and Madelung deformity in a woman with a Xq25→qter deletion

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Madelung deformity (MD) is a congenital anomaly of the wrist with subluxation of the ulna head. MD may be part of Léri-Weill dyschondrosteosis featuring short stature and mesomelic shorting of the limbs caused by SHOX gene alterations. MD may also be present in monosomy X and, more rarely, in other monogenic disorders such as Albright hereditary osteodystrophy in association to brachydactyly.
We report a 48-year-old woman with MD, short stature (148 cm but in her target height) and premature ovarian failure (age at last menses 38 years) in the absence of brachydactyly, other endocrine anomalies, or abnormal skin pigmentation. SHOX gene mutations and rearrangements were excluded by molecular techniques. Conventional cytogenetic analysis demonstrated euploid female karyotype with a deletion of the long arm of one X chromosome in band q24→qter. To better characterize the rearrangement, we performed array-CGH analysis that showed a deletion extending about 33 Mb with breakpoints in Xq25→q28.
Distal Xq deletions are a well-known cause of premature ovarian failure (POF1) but we failed to identify other women with MD and such deletions in the literature. This patient reinforces the concept of genetic heterogeneity in MD suggesting the existence of other, yet unrecognized factors in its pathogenesis, including terminal Xq deletions.

J04.28
Mutations spectrum at autosomal recessive congenital ichthyosis in patients from Russian Federation.

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Autosomal recessive congenital ichthyosis (ARCI) is a heterogeneous group of disorders of keratinization. It characterized by abnormal skin scaling and sometimes it is associated with erythema. It divides on several types the main of which are lamellar ichthyosis (LI) and nonbullous congenital ichthyosiform erythroderma (NCIE), although phenotypic overlap within the same patient or among patients from the same family. Mutations in several genes can lead to both LI and NCIE.
We performed analysis DNA from patients from 40 families on mutations in TGM1 and Alox12B genes. We found mutations in 7 families in TGM1 gene. 7 mutations were novel: p.Trp61Term, p.Val115Met, p.Tyr134His, p.Gly296Arg, p.Pro474Leu, p.Ser691Leu, and one deletion c.566delG. There are no frequent mutations in this gene.
We detected mutations in Alox12B gene in 13 families. There were 6 novel mutations: p.Tyr97Term, p.Ser125Term, p.Gly265Arg, p.Ala412Asp, p.Ser502Asn, p.Asn594His. Two mutations were frequent: p.Ala597Glu at exon 14 was found in 9 families, p.Tyr521Cys at exon 12 was detected in 6 families. Together both mutations were found in 12 families or 92% families with mutations in Alox12B gene.
Thereby among 40 families diagnosis ARCI was confirm for 20 families or 50%: 17.5% families have mutations in TGM1 gene and 32.5% - in Alox12B gene.

J04.29
A novel MSX1 gene splice mutation due to an intronic nucleotide substitution is the cause of congenital tooth agenesis in a Japanese family

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Congenital tooth agenesis is caused by mutations in the *MSX1*, *PAX9*, *WNT10a*, or *AXIN2* genes. Here, we report a Japanese family with nonsyndromic tooth agenesis caused by a novel nucleotide substitution in the intronic region between exons 1 and 2 of the *MSX1* gene. Because the mutation is located 9 bp before exon 2 (c.452-9G>A), we speculated that the nucleotide substitution would generate an abnormal splice site. Using cDNA analysis of an immortalized patient blood cell, we confirmed that an additional 7-nucleotide sequence was inserted at the splice junction between exons 1 and 2 (c.451_452insCCCTCAG). The consequent frameshift generated a homeodomain-truncated MSX1 (p.R151fsX20). We then showed a subcellular localization of the mutant MSX1 in the cytoplasm of transfected COS7 cells, suggesting deletion of the nuclear localization signal, which is mapped to the MSX1 homeodomain. These results indicate that this novel intronic nucleotide substitution is the cause of tooth agenesis in this family. To date, most MSX1 variants isolated from patients with tooth agenesis involve single amino acid substitutions in the highly conserved homeodomain or deletion mutants caused by frameshift or nonsense mutations. We here report a rare case of an intronic mutation of the *MSX1* gene responsible for human tooth agenesis. In addition, the missing tooth patterns were slightly but significantly different between an affected monozygotic twin pair of this family, showing that epigenetic or environmental factors also affect the phenotypic variations of missing teeth among patients with nonsyndromic tooth agenesis caused by an *MSX1* haploinsufficiency.

J04.30

A novel intragenic duplication of FBN1 exons 36-46 in a spontaneously aborted fetus with features of Marfan syndrome.

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Introduction: Marfan syndrome (MFS) is a connective tissue disorder caused by mutations in FBN1. It affects ~1/5,000 people and 75% of cases are inherited. A spectrum of mutations are reported to cause disease; however, to date there have been no reports of large intragenic duplications causing MFS. Here, we report a de novo duplication of exons 36-46 of FBN1 in a fetus with bilateral talipes, enlarged cisterna magna, an unfolded aorta and long, thin, overlapping fingers.

Methods: Sanger sequencing and MLPA of exons 1-65 of FBN1 in the fetal sample, and MLPA analysis of both parental samples were completed.

Results: Sequencing analysis of the entire coding region of FBN1 did not detect any pathogenic mutations. However, FBN1 MLPA analysis detected a heterozygous duplication of exons 36-46. Neither unaffected parent was found to be heterozygous for this mutation, supporting likely pathogenicity of this change.

Conclusion: To our knowledge, intragenic duplications involving multiple exons of FBN1 have not been previously reported in humans. If this is an intragenic tandem duplication it would be predicted to cause a frameshift and therefore would likely be pathogenic.

Although intragenic duplications have not been previously described in humans, the tsk mouse has an intragenic tandem duplication of FBN1 (exons 17-30) and demonstrates cardiac hypertrophy, tight skin and skeletal features associated with MFS (Siracusa et al (1996) Genome Research 6:300-313).

This novel duplication is likely to be the cause of the abnormalities seen in this fetus, and expands the range of pathogenic mutation types detected in MFS.

J04.31

Variants within the COL5A1 3'-untranslated region (UTR) may contribute to the aetiology of musculoskeletal soft tissue injuries

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A polymorphism (rs12722, C/T) within the COL5A1 3'-UTR is associated with musculoskeletal soft tissue injuries. COL5A1 encodes for the α1 chain of type V collagen, an important regulator of fibril assembly. Variants in the 3'-UTR may regulate synthesis of the α1(V) chain and type V collagen production thereby influencing the mechanical properties of the tissues. Functional differences were established between the COL5A1 3'-UTR cloned from participants with a severe chronic Achilles tendinopathy (TEN) phenotype and healthy asymptomatic control individuals. Indeed, two major forms, the C- and T-allelic form, were identified and additional variants were shown to be associated with TEN. To further investigate these functional differences and to start mapping the regions responsible for the tendinopathic phenotype, skin biopsies from donors having a known genotype at rs12722 were taken and primary fibroblast cell lines were established to quantify COL5A1 and COL1A1 expression in a pilot study. Site-directed mutants were generated for two implicated variants, namely rs12722 and rs71746744. Lastly, in preliminary RNA EMSAs, biotinylated C- and T-allelic RNA probes for a 57bp region were incubated with either nuclear/cytoplasmic protein extracts to investigate putative distinguishing RNA:RBP complex formation. An overall higher relative mRNA expression of both COL5A1 ($p<0.001$) and COL1A1 ($p=0.0015$) were observed in the presence of rs12722 TT genotype compared to CC. Although the rs71746744 and rs12722 variants appeared to be functional, they did not independently contribute to the tendinopathic phenotype. However, a unique RNA:RBP complex was identified with the C-allelic probe. These novel results have implications for our understanding of the molecular basis of musculoskeletal soft tissue injuries.

J04.32

NGS panel makes new diagnoses in Ehlers-Danlos syndrome & related HDCT

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Background

Ehlers-Danlos syndrome (EDS) is a significant inherited cause of morbidity and mortality, often presenting at a young age with significant vascular complications. There is wide genetic and phenotypic heterogeneity and overlap with other hereditary disorders of connective tissue (HDCT). The spectrum of mutations in known genes and correlation with phenotype in a mixed cohort of EDS remains to be characterised.

Methods

We used a multiplex-PCR targeted NGS (Fluidigm/MiSeq) assay to sequence the coding regions of COL1A1, COL1A2, COL3A1, COL5A1, COL5A2, FBN1, TGFBR1, TGFBR2, SMAD3, MYLK, MYH11, ACTA2 in a cohort of 177 deeply-phenotyped unrelated patients referred with suspected EDS (mean age 33.8 years, 66% female: 7% Classical, 10%Vascular, 42% Hypermobile, 17% other EDS, 24% other HDCT).

Results

The NGS assay identified 24 pathogenic mutations and 21 variants of uncertain significance (VUS) in the collagen genes and 7 mutations in FBN1, TGFBR1, TGFBR2, SMAD3. Ten high-priority rare variants were found outside the expected genotype-phenotype relationship. Four pathogenic variants (COL3A1, COL5A1, FBN1) and two possible pathogenic VUS (TGFBR2, TGFBR1) represented new diagnoses, not previously detected, in addition to 18 newly identified VUS.

Conclusions

Unbiased targeted sequencing using NGS in combination with current clinical phenotyping can increase the rate of molecular diagnosis in EDS and will enhance our understanding of genotype-phenotype relationships in EDS and other hereditary disorders of connective tissue.

J04.33

Localization of the pathogenic gene of accessory fagus phenotype in a Chinese Han family

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Introduction:To localize the pathogenic gene of Accessory fagus phenotype in a Chinese Han family. Material and Methods: A five-generation family consisting of 56 individuals with or without Accessory fagus phenotype was found in Chinese Han people. Genescan, Linkage analysis and haplotype analysis were used to map the candidate gene by Microsatellite genetic marker.

Results: Genescan and Linkage analysis indicated that the pathogenic gene was located either between d18s462~d18s70, the heretary distance was 6.00cM,LODZMAX =1.83(d18s462,θ= 0.06), or located between d7s2546 and d7s550, the hereditary distance was 8.94cM, LODZMAX =2.74(d7s2546,θ=0.05). Further haplotype analysis narrowed the pathogenic gene between d7s2546~d7s550,the hereditary distance was 5.38cM.

Conclusion:the pathogenic gene of the Accessory fagus phenotype this family was located between d7s2546~d7s550 in chromosomes 7q36.1~7q36.2.

J04.34

Exome sequencing reveals a mutation in DMP1 in a family with familial sclerosing bone dysplasia

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Introduction: Hypophosphatemic rickets (HR) comprises a rare group of inherited diseases. Very recently, mutations in the dentin matrix protein 1 (DMP1) gene were identified in patients with an extremely rare autosomal recessive form of HR (ARHR). To date, very few cases of these mutations were reported.
Materials and methods: A Lebanese consanguineous family with 2 affected sisters was studied. Patients aged 45 and 47 years old presentedwith short stature, severe genu varum, cranial hyperostosis and a very high bone density that led to a diagnosis of a familial sclerosing bone dysplasia. Molecular analysis of known genes involved in osteopetrosis showed normal results. A combination of genotyping and exome sequencing was performed in order to elucidate the genetic basis of this pathology.
Results: Biochemical analysis was consistent with normal serum calcium and 1-25(OH)2D levels, low to normal serum phosphorus and elevated PTH values. Serum c-terminal FGF-23 was elevated in one of the two patients. A homozygous mutation disrupting the initiation codon of the DMP1 gene (OMIM 600980), NM_001079911.2: c.1ANG, p.Met1Val, was identified by exome sequencing and confirmed by Sanger sequencing.
Conclusion:We report here a family of ARHR secondary to aDMP1mutation located in the first coding exon of the gene. Our cases show that some ARHR cases may develop with age an unaccountable increase in bone density and bone overgrowth.

J04.35

New clinical presentation of tooth agenesis associated with p.Phe228Ile WNT10A mutation

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Tooth development is under strict genetic control and mutations of genes regulating early stages of tooth formation have been found to cause tooth agenesis, i.e. congenital absence of teeth.
In our study we sequenced the WNT10A, PAX9, AXIN2 and MSX1 genes in a patient presenting agenesis of mandibular and maxillary second premolars in order to identify potential mutations. The patient carries a heterozygous missense mutation c.682T>A (p.(Phe228Ile)) in the WNT10A gene. This mutation is predicted to be damaging with a Polyphen score of 0.999 and a Global MAF of 0.010 (allele A). This coding variant has previously been reported in 2 patients with odonto-onychodermal dysplasia and in one family with variable hypodontia involving lateral incisors and premolars. Another study revealed that this variant is the most prevalent in the WNT10A gene and represent13% of all oligodontia alleles detected in their cohort.
In our study, we report a novel dental phenotype associated with the (p.(Phe228Ile)) coding variant in the WNT10A gene involving agenesis of all second premolars. In the absence of other systemic anomalies, and based on current insights into the interacting signaling pathways at the initiation stage of tooth development, we hypothesize that this new phenotype might be caused by additional polymorphisms observed in the PAX9, AXIN2 or MSX1 genes.

J05.01

Prevalence of CYP2C8*3 and CYP2J2*7 in Bulgarian patients with coronary artery disease and hypertension

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The cytochrome P450 epoxygenases are important enzymes metabolizing arachidonic acid to different vasoactive metabolites.
We conducted a case-control study to determine the prevalence of polymorphic variants in epoxygenase - related genes CYP2C8 (rs1050968; CYP2C8*3) and CYP2J2 (rs890293; CYP2J2*7) in the Bulgarian population and to evaluate whether these genetic variants are associated with increased risk of coronary artery disease (CAD) and /or essential hypertension (EH) in the studied cohort.
The current analysis included 192 unrelated hypertensive patients, 261 patients with angiographically documented CAD (153 with myocardial infarction and 108 without myocardial infarction) and 496 population - based controls. The CYP2C8*3and CYP2J2*7 polymorphisms were genotyped by TaqMan SNP Genotyping Assay (Applied Biosystems). PLINK version 1.07 was used for the statistical analysis.
The frequency of -50T mutant allele of CYP2J2*7 was significantly higher in male with angiographically documented CAD without history of myocardial infarction (OR 2.16 95%CI 1.04-4.48 p=0.035) compared to population control group, but this association did not survive after Bonferroni correction (padj=0.07). A significant association of a CYP2C8*3 allele with increased risk of EH was found in men (OR 2.12 95%CI 1.18-3.81 p=0.015) and this relationship remained significant after adjustment for multiple comparisons (padj=0.02).
In conclusion, these results could be due to the great impact of sex hormones such estrogens on the vascular tone and CYP2J2/CYP2C8 gene expression. Therefore, the protective effect is more evident in females compared to males.
This work was supported by DUNK01/2/28.12.2009, funded by NSF, Ministry of Education, Youth and Science and Grant № D-1/2011-MU Pleven; Bulgaria

J05.02

Distribution of angiotensin-I converting enzyme insertion/ deletion and α-actinin- 3 codon 577 polymorphisms in Turkish male soccer players

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Angiotensin-1 converting enzyme gene (ACE) and α-actinin- 3 gene (ACTN3) polymorphisms are considered to be the most important candidate genes for genetic predisposition to human performance . In the present study, we aimed to analyze the distribution of ACE and ACTN3 polymorphisms in male Turkish soccer players In this prospective study, our cohort consisted 25 professional players, all with Turkish ancestry. Genotyping protocol were carried out by polymerase chain reaction- restriction length polymorphism (PCR- RFLP) for ACTN3 and PCR for ACE. 16%, 44% and 40% of the players had II, ID and DD genotype, respectively, for ACE genoype; whereas 20% had XX, %36 had RX and %44 had RR genotypes for ACTN3. When we examined the allelic percentages, for ACE, D allele was recorded as 62 and I as 38; and for ACTN3, R allele was 62 and X was 38. Our results were in agreement with the previous reports, indicating the presence of D allele and X allele in soccer players. We suggest that ACE and ACTN3 genotypes are important biomarkers for genetic counseling for the individuals who are prone to be successful in soccer.

J05.03

Genetic tests help to choose a therapeutic step with high complication rate in a family with hypertrophic cardiomyopathy: a case report.

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Genetic testing is becoming a more valuable tool in clinical decision making. Here, we discuss the case of a family with hypertrophic cardiomyopathy where the generally used diagnostic approaches did not give enough information for a correct decision of implanting an implantable cardioverter defibrillator (ICD). A 7 year old female child was transported to a critical care center after a short reanimation which was carried out after she had collapsed without prodromal signs during a regular physical activity. The father had typical MRI signs for HCM while the mother's MRI did not show any change characteristic for HCM. The sudden cardiac death together with the diagnosed HCM indicated the implantation of ICD. The detailed analysis of Holter, ECG and MRI did not show typical signs that could have indicated ICD implantation for the 2 older sisters. Genetic testing was offered to the family and we have identified two disease-causing heterozygous mutations in the MYBPC3 gene (p.R495Q and p.S593fs*11) in the children. This information finally helped to make the decision of implanting ICD into both sisters as primary prevention, despite of the associated average of 25% complication rate of the procedure in children. Later, the father (carrying the p.R495Q mutation) also received an ICD after a 30 second collapse with unknown origin. Since the mother (carrying the p.S593fs*11 mutation) - for the time being - does not have any morphological or clinical sign of HCM, ICD implantation was not indicated in her. This case further supports the importance of genetic testing in clinical practice.

J05.04

Compound and individual effect of heterozygous missense mutations in long QT syndrome

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Congenital long QT syndrome (LQTS) is a rare heart disorder that is characterized by prolonged QT interval, syncopes, seizures and/or sudden death. Up to now, 13 LQTS genes have been identified.

We have developed a gene panel for targeted resequencing of the 13 genes based on AmpliSeq™ technology. The panel covers coding exons and adjacent 5 bp parts of introns. We performed clinical and genetic characterization of two unrelated LQTS family trios. The first proband (child in family #1) harbours 2 missense mutations inherited from his mother and father, respectively: KCNH2 c.526C>T, p.(R176W) and KCNQ1 c.775C>T, p.(R259C); the second proband - KCNH2 c.526C>T, p.(R176W) and KCNE1 c.253G>A, p.(D85N) from his mother and father. Both patients suffer severe LQTS with significantly prolonged (>500 ms) QT intervals and have cardioverter-defibrillator implanted.

Effect of the KCNH2 mutation (rs36210422) found in both probands is known to be controversial. Here, we report two phenotypically healthy women harbouring this mutation (with normal QTc). Pathogenicity of the KCNE1 mutation (rs1805128) is also known to be controversial. Here, we report a phenotypically healthy man harbouring the mutation with slightly prolonged QTc (~450 ms). The KCNQ1 mutation (rs199472719) that affects the structure of an evolutionally conservative intracellular linker of S4-S5 protein domains and is reported to be pathogenic by multiple authors also demonstrates no strong phenotypic effect itself (QTc ~450 ms in father of proband #1).

Our results support the concept of severe effect of compound heterozygous mutations in LQTS with the effect of solitary mutations being usually ambiguous.

J05.05

Mitochondrial DNA control region heteroplasmy in carotid atherosclerotic plaques and blood cells

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Atherosclerosis is characterized by increased oxidative stress which can induce DNA damage. Mitochondrial DNA is particularly subjected to oxidative damage. So mtDNA mutations in somatic cells could be a result of oxidative stress and, in turn, may lead to uncoupling of oxidative phosphorylation through their effect on mtDNA-encoded proteins (further stress increasing). To study possible somatic mutations in mtDNA during atherosclerotic plaque development, we sequenced control region of mtDNA (16024-400) in 24 patients who have undergone surgery on carotids. DNA was isolated from the plaques and blood leucocytes. Sanger sequencing (allowing detection of 10% heteroplasmy level) was performed on ABI 3730 analyzer. In total, we have detected 5 heteroplasmic transitions in 4 individuals (16.7%). In addition, common heteroplasmy in polycytosine tracts 16184-16193 and 302-309 has been registered in 17% and 70% patients, respectively. While comparing plaque and blood samples for each patient, 3 of the heteroplasmies were common for the plaque and blood of the individuals, whereas two heteroplasmic positions were detected only in the plaques, so they could be result of somatic mutations. The results support the assumption that atherogenesis is accompanied by mtDNA damage.

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J05.06

Molecular analysis of the BMPR2 gene in patients with Pulmonary Arterial Hypertension: preliminar data of an Italian study

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Pulmonary arterial hypertension (PAH) is a rare disorder of the small pulmonary arteries that determines proliferative and fibrotic changes characterized by progressivity, leading to vessel blockage, right heart failure and death. The major gene associated to PAH, according to an autosomal dominant inheritance pattern, is BMPR2 that encodes the 'bone-morphogenetic-protein-receptor, type II (serine/threonine-kinase)'. At our Cardiology Unit, a Center of Excellence for diagnosis and therapy of PAH, within the project concerning the BMPR2 screening in PAH patients, to date, we (LS, SG) enrolled 30 probands. The BMPR2 analysis by direct sequencing of the 13 coding exons has been completed in 26 (19 female): 6 (23%) patients, 3 (43%) male and 3 (16%) female, showed a private mutation; these nucleotide changes were absent in dbSNP141, 1000Genomes and Exome-Variant-Server dataset. In four patients a BMPR2-haploinsufficiency is predictable, because the mutation leads to a premature termination codon; in other two were identified the mutations p.(Cys117Gly) (not described) and p.(Arg491Gln) (Deng Z et al., 2000) that, by use of Blastp, have been located within the consensus CCX{4-5}CN (hydrophilic-cysteine-rich-ligand-binding domain) and in the 'Catalytic domain of the Serine/Threonine-Kinases', rispectively. The cysteine substitution in the ligand-binding domain is plausibly causative of retention of the protein in the endoplasmic reticulum (Rudarakanchana N et al., 2002). This abstract describes preliminary data of the first Italian study aimed to characterize the genetic basis of PAH in a large number of patients; this will ensure a more focused and effective clinical management of PAH patients together with the goal to move toward application of personalized medicine.

J05.07

Desmosomal gene mutations in dilated and arrythmogenic cardiomyopathy

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Desmosome proteins defects are associated with right ventricular (fibro)-fatty replacement of myocardial tissue (Arrythmogenic Right Ventricular Cardiomyopathy/Dysplasia, ARVC/D, MIM #609040 and #107970). The causative genes are: JUP (17q21), PKP2 (12p11), DSC2 (18q12.1), and DSG2 (18q12.1-q12.2), DSP (6p24), TGFB3 (14q24.3), TMEM43 (3p25.1), CTNNA3 (10q21.3). We screened the eight genes in a consecutive series of 45 ARVC/D patients and 25 DCM patients with arrhythmias. The corresponding mean age was 42.3±11.3 years and 45± 8.7 years. The ARVC/D was diagnosed on McKenna et al. criteria, the DCM according to the WHO criteria. The selected genes were analysed by targeted NGS (PGM, Life Technologies). We identified 54 mutations in 70 patients (30 of the 45 ARVC/D patients and 24 of the 25 DCM patients), with 12 patients carrying a double/compound heterozygous mutation and 6 patients carrying a triple mutation. The segregation of the gene defects with the phenotype in the family was confirmed in 3 probands (who fulfilled the McKenna criteria of ARVC/D). The recurrence of double/triple mutants in these patients raise serious problems in molecular diagnosis and family study, in particular the prediction of the development of the disease in young mutation carriers. The desmosome gene defects associated phenotypes include DCM with ventricular arrhythmias. The screening of the candidate genes should not stop when finding a mutation and the segregation of the phenotype with the defect is essential to help the correct interpretation of the molecular genetic data. Due to the current uncertainties, healthy mutation carriers should undergo

regular clinical monitoring.

J05.08

Association of allelic polymorphisms of matrix Gla protein system genes with acute coronary syndrome in the Ukrainian population

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Background - The matrix Gla-protein (MGP) has an important role in vessel protection against ectopic calcification; its presence in tissues prevents initiation and spread of a pathological calcification. It gave a reason to talk about MGP functional system that yields, except for the MGP protein, such factors as vitamin D receptor, enzymes involved in biochemical transformations of MGP, vitamin K epoxide reductase and vitamin K-dependent gamma-glutamyl carboxylase, including bone morphogenetic protein-2.

Methods - The association between acute coronary syndrome (ACS) and 10 polymorphic variants of MGP-system genes was analysed. Venous blood of 118 patients with ACS and 234 healthy individuals was used for genotyping. Polymorphisms of MGP-system genes were examined with PCR-RFLP methodology.

Results - The risk of ACS in carriers of minor allele A/A (G-7A) in 2.8 times higher; B/B (BsmI) in 2.1 times higher; Gln/Gln (Arg325Gln) and C/C (T2255C) twice higher than in carriers of the major allele. The best classification model is a two-component model that includes polymorphisms MGP gene G-7A and Thr83Ala. The coincidence of similar orientation genotypes variants for chosen polymorphism was associated with a high risk of developing ACS.

Conclution - There is an association between the ACS and some polymorphic variants of genes of MGP system.

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J05.09

ROS1, TAS2R50, ZNF627, MIAF3 genes, 16q23.1, 6p24, 9p21 chromosomal regions and myocardial infarction in siberia caucasians

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The purpose: aim of the study to test SNP identified in recent genome-wide association studies on the suitability as risk markers of myocardial infarction in the Siberian population Caucasians.

Materials and Methods: The study had taken two groups: myocardial infarction 275 people and a control group 420 people, comparable in sex and age differences are formed on the basis of population sample of 45-69 year old residents of Novosibirsk, established under the international project HAPIEE. Genomic DNA was isolated from venous blood by phenol-chloroform extraction. Gene polymorphism was tested by real-time PCR according to the protocol of the manufacturer (probes TaqMan, Applied Biosystems, USA) on the instrument ABI 7900HT. The study included the following single nucleotide polymorphisms: rs499818, rs619203, rs10757278 and rs1333049, rs1376251, rs2549513, rs4804611, rs17465637.

Summary of the results: For rs1376251, we have shown that the CC genotype was associated with MI OR = 1.37 (95% CI 1.005-1.888 CCvs. CT + TT p = 0.05). For rs499818 was shown that the AA genotype has a protective effect OR = 0.3 (95% CI 0.103-0.883 AAvs. AG + GG p = 0.025). Considering rs10757278 genotype GG shown predisposing OR = 2.1 (95% CI 1.47-3.05, p = 0.001), and CC genotypes in rs1333049 CC OR = 2.1 (95% CI 1.47-3.05, p = 0.001)

Conclusions: We identified a significant association between rs1376251, rs499818, rs10757278, rs1333049 and myocardial infarction.

J05.10

The molecular genetic markers of myocardial infarction and sudden cardiac death in a Russian population.

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Motivation and Aim: Recent genome-wide studies have detected the association of some single nucleotide polymorphisms (SNPs) with an increased risk of myocardial infarction. According to ICD-10, sudden cardiac death (SCD) does not include the death of myocardial infarction; however, the clinical picture and the mechanism of fatal outcome development in sudden cardiac death and myocardial infarction are rather similar. So the aim of this work is investigate the association of rs17465637 gene MIAF3 (1q41), rs1376251 gene TAS2R50 (12p13), rs4804611 gene ZNF627 (19p13), rs619203 gene ROS1 (6q22), rs1333049 (9p21), rs10757278 (9p21), rs2549513 (16q23), rs499818 (6p24) associated with myocardial infarction available from the international genome-wide studies with SCD in a case study of a Russian population.

Methods and Algorithms: A sample of SCD cases (n = 285) was formed using the WHO criteria; the control sample (n = 421) was selected according to sex and age.

Results: No statistically significant differences in the genotype and allelic frequencies of rs17465637, rs2549513, rs1376251, rs4804611, and rs619203 polymorphisms between SCD cases and control were detectable. Genotypes CC of rs1333049 and GG of rs10757278 are associated with an increased SCD risk in men (p=0.019, OR=1.7, 95%CI 1.1-2.8; p=0.011, OR=1.8, 95%CI 1.2-2.8, respectively). Genotype AG of rs499818 is associated with an increased SCD risk in the women over 50 years old (p=0.009, OR=2.4, 95%CI 1.3-4.6).

Conclusion: Polymorphisms rs1333049 and rs10757278 are associated with SCD in men and rs499818 in the women aged over 50 years.

J05.11

Molecular-genetic analyses of single nucleotide polymorphism in the ZBTB17 gene in Slovak patients with dilated cardiomyopathy

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Dilated cardiomyopathy (DCM) is a primary myocardial disease and genetically heterogeneous disorder. It represents a major cause of cardiovascular morbidity and mortality and is characterized by systolic dysfunction, dilation, and impaired contraction of the ventricles, often leading to chronic heart failure and eventually requiring cardiac transplantation. Mutations in both sarcomeric and cytoskeletal genes have been implicated in DCM. Recently, a GWAS study on DCM indicated that rs10927875 single nucleotide polymorphism (SNP) in ZBTB17 gene was associated with DCM. The aim of the study was to analyze the distribution of SNP rs10927875 in ZBTB17 gene in 150 Slovak subjects, 55 patients with DCM (51.7±6.9 years) and 95 healthy controls (51.7±6.9 years) using the Custom Taqman®SNP Genotyping assays. The distribution of ZBTB17 gene rs10927875 polymorphism in Slovak patients with DCM was as follows: CC (36.4%), CT (58.2%), TT (5.4%), in controls: CC (44.2%), CT (51.6%), TT (4.2%). There was no difference in genotype or allele frequencies in ZBTB17 gene rs10927875 polymorphism (OR=0.75, 95% CI=0.46-1.23, P=0.25) between DCM patients and control subjects. Further studies in numerous files and additional functional investigations are needed to fully understand the roles of genetic associations. Knowledge of the genetic risk factors for DCM is important to initiate treatment prior to symptomatic onset of the disease to delay its occurrence or possibly halt its progression.

This study is the result of implementation of the project APVV-0644-12.

J05.12

GENES KIAA1462, CDKN2BAS1, LIG1, ADAMDEC1, APOA2 IN PREDISPOSITION TO MYOCARDIAL INFARCTION

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A set of SNPs in genes related to fibrogenesis and cardiovascular diseases was investigated in the sample of patients with myocardial infarction (IM), which were collected in Tomsk and Kemerovo cities in West Siberia (Russians). The patients were divided into two subgroups according to presence or absence of co-existing diseases: (1) patients with IM and without other diseases of cardiovascular continuum (hypertension, hypercholesterolemia, type 2 diabetes mellitus) - "isolated IM" (n=60); (2) patients with IM and all

the pathologies, mentioned above - "syntropy of cardiovascular disease continuum" (syntropy CDC) (n=96). As a control, population sample of Tomsk residents (n=300) was considered. Altogether, 58 SNPs were genotyped by mass-spectrometry (Sequenom MassARRAY).
Comparing genotype frequencies between patients and controls, we found following significant associations: (1) rs1333049 in CDKN2BAS1: allele C (OR=1.35 (1.00-1.82), p=0.024); CC+CG vs GG (OR=1.68 (1.00-2.83), p=0.047); (2) rs20579 in LIG1: allele C (OR=1.78 (1.11-2.81), p=0.010); CC vs CT+TT (OR=1.69 (1.00-2.72); p=0.009); (3) rs3739998 in KIAA1462: allele C (OR=1,66 (1.23-2.23), p=0.001); CC vs CG+GG (OR=2.46 (1.48-4.08), p=0.0002); (4) rs5082 in APOA2: allele T (OR=1.45 (1.07-1.96), p=0.015), TT vs TC+CC (OR = 1.76 (1.13-2.70), p=0.032).
Association analysis in the subgroups of patients has revealed differences in the genetic predisposition to the isolated IM and syntropy CDC. The KIAA1462 (rs3739998) association is common for both groups. LIG1 (rs20579) and ADAMDEC1 (rs3765124) are associated with the isolated IM. CDKN2BAS1 (rs1333049) and APOA2 (rs5082) are associated with the syntropy CDC.

J05.13
The frequencies of mutation in the prothrombin (FII G20210A) gene and factor V Leiden (FVL) in the Russian endurance elite athletes.

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Endurance athletes represent a group of risk of arterial and venous thromboses. Cases of coronary thrombosis in marathon, VTE in triathlon were described (Albano AJ, 2012; Tao K, 2010; Hull CM, 2014). It is caused by several factors: dehydration with haemoconcentration, pharmacological stimulators, injuries, immobilization at traveling, etc. The role of a hereditary thrombophilia at this contingent is studied a little.
PURPOSE. To investigate the frequencies of FII 20210A and FVL mutations in sample of Russian endurance athletes.
METHODS. 559 unrelated Caucasians were studied. Athletes: 264 persons, age: 26,3±10,3 years (±SD), including 152 ice hockey players, 62 biathlonists, 18 triathlonists, 14 walkers, 3 runners, one cyclist. 87 athletes were winners of the international competitions (elite). Sedentary control: 295 persons, age of 31,2±10,4 years. All participants gave the written informed consent. Biological samples: buccal epithelium. gDNA was extracted by sorbent method. Genotyping: TaqMan® SNP Genotyping Assays, StepOne™ Real-Time PCR System (LifeTechnologies, USA).
RESULTS. All participants had no the burdened thrombotic anamnesis. Pathological homozygotes were absent. Frequencies of heterozygotes (±95% confidence interval) are present in Table.

	FII 20210A	FVL
Controls	2,4±1,7	1,7±1,5
Athletes, all	3,0±2,1	4,5±2,5
Athletes, elite	6,9±5,3	4,6±4,4

Frequencies of heterozygotes didn't differ significantly between groups of athletes (pooled and elite level) and controls. Aerobic conditions accompany endurance sports, stimulate fibrinolysis and provide antithrombotic effect.
CONCLUSIONS. Observed frequencies of FVL and FII 20210A in sample of Russian endurance athletes didn't differ from sedentary control. Endurance sports didn't increase thrombotic risk for young carriers of FVL and FII 20210A heterozygotes.

J05.14
ACE Gene I/D Polymorphism in the Czech population

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Angiotensin converting enzyme (ACE) is a part of RAAS system, which regulates blood pressure, management of water and minerals and has behavioral effects (thirst, taste, memory and learning regulation). The enzyme is coded by ACE gene, the most frequent Polymorphism I/D determinates the presence of insertion (I) or deletion (D) of 287bp Alu repeat sequence in intron 16. This polymorphism regulates plasma level of enzyme which decreases from DD to II genotype. The aim of our study was to determinate allele/genotype frequencies of this polymorphism in Czech population.
940 individuals (470 F, 470M, age 18-69 years) of the Czech population were tested. DNA was isolated from oral mucosa or blood using MagCore HF16. ACE Gene I/D Polymorphism was tested by the End-point PCR and agarose electrophoresis with visualization under UV light, verification was performed by strip assay reverse hybridization method.
Allele I was detected in 47,2%, allele D in 52,8% of samples. The frequency of genotypes: II - 21,8% (21,3% F, 22,6% M), ID- 50,8% (51,7% F, 49,5% M) a DD- 27,4% (27,0% F, 27,9% M).
According to published data of the European populations, the Czech population with slight predominance of allele D and risk DD genotype (+5,6 %) surprisingly correlates with Hungarian and Turkish population, while it differs from the Polish population. Because there are significant differences among populations, it is important that the control group of Czech population was made for further genetic studies of this polymorphism in relation to diseases and pathological conditions of Czech patients.

J05.15
Prevalence of NOTCH1 signalling pathway mutations in subjects with bicuspid aortic valve

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The bicuspid aortic valve (BAV) (OMIM #109730) is the commonest congenital cardiac malformation, occurring in ~2% of the population. Missense mutations in the NOTCH1 gene have been shown to be associated with calcific aortic valve disease plus BAV. However mutations in the NOTCH1 gene only partially explain the presence of BAV in the absence of other syndromic features (i.e. Marfan Syndrome, Loeys Dietz Syndrome type I and II and Ehlers Danlos type IV Syndrome). In this study we analysed 50 patients with true BAV assessed by cardiac imaging (none of them with syndromic feature), for mutations in genes (n=48) belonging to the NOTCH1 signalling pathway (http://pathcards.genecards.org/pathway/1766) by means of massive parallel sequencing (My Seq, Illumina). In 35 patients (70%), we identified novel de novo mutations in NOTCH1, 15 (30%) patients inherited rare gene variants in FOXC2, FOXL1, PSEN2, ADAM17, DTX2 and DTX4. Some of the identified variations (n=26, 52%) coexisted in the same patient. The biological significance of such rare variations is unknown, but our findings strengthen the role of NOTCH pathway in cardiac valve development, indicating that BAV is, at least in part, an inherited familial disorder. This report strengthens the use of massive parallel sequencing to provide insights in common disorders. Moreover, the coexistence of multiple rare variants suggests in some cases a cumulative effect, as shown for other complex disease.

J05.16
Alport syndrome and cardiovascular abnormalities. D. Shentseva, O. Groznova, L. Shagam, A. Polyakova, V. Sukhorukov, N. Konkova, V. Dlin. Pirogov Russian National Research Medical University (RNRMU).

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Alport syndrome (AS) is a genetically determined glomerulopathy that is caused by mutations in type IV alpha collagen chain genes (COL4A3, COL4A4or COL4A5) and clinically characterizedby hematuria, proteinuria andend-stage kidney disease (ESRD). The syndrome is often combined with sensorineural hearing loss and ocular pathology.
We examined 21 children with Alport syndrome, the diagnosis was verified by molecular genetic studies. In 83% cases the mutation COL4A5 was determined, and X-linked version of the SA was verified.
By echocardiography we observed in children with X-linked AS up to 9 years of age dimensions of the aortic root and fibrous ring of the aortic valve does not exceed the normative values (up to 95 percentile according to body surface area). In older children (10-17 years) in 62% of cases of aortic root dilatation, followed by dilation of the aortic annulus and diastolic aortic valve prolapse.
The of the aorta in Alport syndrome is still poorly understood. To date, there is no doubt that a significant proportion of patients have manifestation of vascular disease.

J05.17
Hypoplastic left heart syndrome in a case with partial 18p monosomy and partial 20q trisomy

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Chromosomal deletions and duplications have been associated with a variety of congenital cardiac malformations. We report on a patient with prenatally diagnosed congenital left ventricular hypoplasia syndrome, born to healthy non-consanguineous parents with complicated family history (two

miscarriages). After surgery for the condition, bradycardia suddenly developed leading to a fatal outcome on 3rd day of his life. The following dysmorphic features were present in the patient: frontal bossing, prominent nasal bridge, thin lips, oversized ears, left preauricular pit, ear lobe creases on both ears, a single transverse palmar crease on the left palm). X-ray showed thoracic hemivertebrae and 13 rib pairs.
Chromosome analysis of peripheral blood lymphocytes of proband revealed normal karyotype. Whole genome genotyping analysis of the patient showed an unbalanced translocation of paternal origin: a deletion in the region 18p11.32 - 18p11.31 (12,842-4,652,432) and a duplication in the region 20q13.2 - 20q13.33 (50,208,036-62,909,908) of 4.6 Mb and 12.7 Mb in size, respectively. The clinical cardiac characteristics of patient could be associated with combination of both chromosomal aberrations, containing 27 OMIM annotated genes in total. Combination of genes in these regions could contribute to the development of the cardiac malformation in our patient.

J05.18

Multidisciplinary diagnostic workup in cardiovascular patients

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Since February 2012 we recruited a cohort of 380 cardiovascular patients from the Medical Genetic Unit and the Department of Cardiology of Niguarda Ca' Granda Hospital of Milan (Italy) in a multidisciplinary diagnostic workup including cardiologic evaluation and genetic counseling.
After clinical and instrumental evaluation all patients received a genetic counselling and signed an informed consent to the genetic study; we tested 18 gene by Sanger sequencing analysis
We report here a summary of the results: 109 patients presented Hypertrophic cardiomyopathy (HCM), 71 Dilated cardiomyopathy (DCM), 47 Arrhythmogenic Right Ventricular cardiomyopathy (ARVC), 16 Fabry disease, 37 Marfan syndrome, 19 Loeys-Dietz, 20 Noonan/Leopard syndrome, 15 Left/Right Ventricular non-Compaction (LVNC/RVNC) and 46 Channelopathies (35 Brugada and 11 Long QT).
In HCM affected subjects we found causative mutation as follows: 24,7% MYBPC3, 9,1% MYH7, and 1,8% TNNT2 genes; in DCM patients: 19,7% in LMNA and 2,8% in SCN5A genes; in Brugada patients: 28,5% SCN5A gene; in LQ patients: 18%mutations in KCNH2 gene; in ARVC patients: 31.9% in PKP2 and 2.1% in JUP genes; in Marfan patients: 27% in FBN1 and 2.7% in TGFBR2 genes; in Noonan /Leopard syndrome patients: 25% in PTPN11gene and in LVNC patients: 13.3% in LDB3 gene.
Search for causative mutations is an integral part of the cardiomyopathy diagnosis. The finding of a causative mutation in a patient permits the identification of family members who may have the disease in an asymptomatic way or who may develop disorders in the future and may in turn transmit the mutation to their offspring.

J05.19

Association of VDR gene polymorphisms with hypertension in patients with atherothrombotic ischemic stroke in Ukrainian population

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Background - Recently genes, which depend on the intensity and direction of the calcium-phosphorus metabolism in the body as a whole and in certain tissues, have been named among factors that influence the damage of blood vessels. These includes vitamin D receptor gene (*VDR*). Furthermore, according to clinical trials, there is an inverse correlation between low level of vitamin D and factors, such as blood pressure (BP), coronary atherosclerosis and various cardiovascular diseases, arterial hypertension in particular.
Materials and methods - Venous blood of 170 patients with atherothrombotic ischemic stroke (AIS) and 124 healthy individuals (control group) was used for genotyping. Four polymorphisms (*Fok*I, *Bsm*I, *Apa*I, *Taq*I) of gene *VDR* were examined with PCR-RFLP methodology.
Results - The association between genotypes of polymorphic variants *Fok*I, *Bsm*I, *Apa*I and *Taq*I *VDR*-gene with the development of atherothrombotic ischemic stroke in people with normal and high blood pressure was found (Table).

Table - The distribution of individuals with different genotypes for polymorphic variants of *VDR* gene in the control group and patients with AIS depending on the blood pressure

BP	*FokI*		*BsmI*		*ApaI*				*TaqI*	
	F/F		*b/b*		*a/a*		*a/A*		*T/T*	
	Control group	Stroke case	Control group	Stroke case	Control group	Stroke case	Control group	Stroke case	Control group	Stroke case
BP(-) (*n*)	16 (48.5%)	6 (15.0%)	23 (41.1%)	16 (22.5%)	17 (43.6%)	7 (15.6%)	21 (42.0%)	22 (25.9%)	21 (39.6%)	15 (22.1%)
BP(+) (*n*)	17 (51.5%)	34 (85.0%)	33 (58.9%)	55 (77.5%)	22 (56.4%)	38 (84.4%)	29 (58.0%)	63 (74.1%)	32 (60.4%)	53 (77.9%)
χ^2	9.629		5.055		8.046		3.768		4.396	
P	0.002		0.025		0.005		0.052		0.036	

Note: n – the number of people, BP(-) – normal blood pressure, BP(+) – high blood pressure, P – level of significance by χ^2 test.

Conclusion – In patients with genotype F/F, b/b, a/a, a/A, and T/T polymorphic variants *Fok*I, *Bsm*I, *Apa*I, *Taq*I of *VDR* gene the association between presence of hypertension and development of atherothrombotic ischemic stroke was established.
Grant reference: The study was a part of the scientific project "Association ectopic calcification genes polymorphisms with widespread cardiovascular diseases and their complications" supported by the Ministry of Education and Science of Ukraine, 2013-2014 (No 0113U000132).

J05.20

Mutations in *TAX1BP3* cause Dilated Cardiomyopathy with Septo-Optic Dysplasia

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We describe a Bedouin family with a novel autosomal recessive syndrome characterized by dilated cardiomyopathy and septo-optic dysplasia. Genetic analysis revealed a homozygous missense mutation in *TAX1BP3,* which encodes a small PDZ-containing protein implicated in regulation of the Wnt/β-catenin signaling pathway, as the causative mutation. The mutation affects a conserved residue located at the core of TAX1BP3 binding pocket and is predicted to impair the nature of a crucial hydrophobic patch, thereby interrupting the structure and stability of the protein, and its ability to interact with other proteins. *TAX1BP3* is highly expressed in heart and brain and consistent with the clinical findings observed in our patients, a knockdown of *TAX1BP3* causes elongation defects, enlarged pericard and enlarged head structures in zebrafish embryos. Thus, we describe a new genetic disorder that expands the monogenic cardiomyopathy disease spectrum and suggests that *TAX1BP3* is essential for heart and brain development.

J05.21

Exome sequencing in sporadic cases of hypoplastic left heart syndrome identifies de novo protein-altering mutation in new candidate genes

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Hypoplastic left heart syndrome (HLHS) is one of the most common and lethal congenital cardiac anomalies, characterized by an underdeveloped left ventricle, mitral valve and aorta. Despite strong evidence of an underlying genetic aetiology, only a minority of cases can be explained on genetic bases. Using genomics as well clinical studies, our work aims to identify new genes contributing to HLHS pathogenesis and explaining clinical and anatomical variability. We identified by exome sequencing novel de novo mutations in genes not previously associated to HLHS. In silico studies suggested that these genes are potentially involved in cardiac morphogenesis, and molecular 3D modeling highlighted the potential pathogenic effect of the identified mutations. Furthermore, we performed in vitro functional characterization of one of these variants falling in low density lipoprotein receptor-related protein 6 (LRP6) gene. The preliminary results suggest that the mutation in LRP6 impairs the WNT signaling required in the induction of endothelial to mesenchymal transition (EndMT) during ventricular chambers formation. Further studies are required to confirm the mechanism by which LRP6 is involved in HLHS pathogenesis.
In conclusion, due to genetic heterogeneity and phenotypic variability, elucidating the causes of HLHS remains challenging. The development of this multidisciplinary approach will help to better understand HLHS by uncovering the multiple genetic mechanisms. Others patients are needed to find new genes.
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J05.22

Complex analysis of predisposition to essential hypertension: differential expression and multigene associations of chemokine genes

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Essential hypertension (EH) is a complex disease that arises from an interaction between environmental and genetic factors. It is been hypothesized that systemic inflammation plays a role in the pathogenesis of hypertension. We aimed to investigate expression profiles of inflammatory mediator genes in patients with EH and to analyze an association between these genes and the development of hypertension.

We performed the analysis of gene expression in peripheral blood leukocytes of patients with EH (N=57) and healthy individuals (N=51) using microarray technology (RT2ProfilerTM PCR Array, SABiosciences Corporation, Qiagen) with subsequent validation of the obtained results by quantitative real-time RT-PCR. The results have confirmed significant differences in transcriptional activity of CCL2, CCL8, CCL18, CX3CR1, CXCL1, CXCL13, CCR5, IL10, IL13, XCR1, and CCR2 genes in cases and controls (p=0.001).

Next, we performed genotyping of polymorphic loci in genes with altered expression profile in the group of patients with EH (N=216) and healthy individuals (N=314). Allelic combinations associated with the disease were identified using Markov chain Monte-Carlo approach (APSampler).

We identified three allelic combinations of chemokine and chemokine receptor genes associated with the increased risk of EH: CCL2 *T+CCR2*I+CX3CR1*M+CCR5*I+CXCR2*T (OR=6.342, FDR=0.04); CCR2*I+ CX3CR1*M+CXCL1*G+CXCR2*T (OR=4.48, FDR=0.04); CCR2*V+CXCL8*A+CCR5*I+ XCR1*T/T (OR=3.11, FDR=0.04).

The results of our study suggest that chemokines and chemokine receptors may play a role in the development of essential hypertension, and demonstrate that the interplay of the polymorphic loci may influence susceptibility to the disease.

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J06.01

Role of peroxisome proliferator-activated receptor- γ coactivator -1 α gene polymorphisms and association of type II diabetes mellitus in south Indian population

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Background In the present study we aim to investigate if variability in the peroxisome proliferator-activated receptor-gamma coactivator-1(PGC-1) gene is associated with Type II (non-insulin-dependent) diabetes mellitus. The objective of study was to explore the relationship between SNPs of PGC-1 α and type 2 diabetes in the south Indian population

Methods The SNPs in all exons of the PGC-1 gene was investigated in 50 type II diabetic patients using PCR-single strand conformational polymorphism (PCR-SSCP) .183 type II diabetic patients and 195 healthy controls were genotyped by restriction fragment length polymorphism -polymerase chain reaction (RFLP-PCR).

Results Three frequent SNPs (Thr394Thr, Gly482Ser and Thr528Thr) were found in exon of the PGC-1α gene. Only the Gly482Ser variant had a different distribution between diabetic patients and healthy subjects, with the 482Ser allele more frequent in patients than in controls(40.1% vs 29.3% P <0.01) .In controls ,the 482Ser(A) carriers were more likely to have higher levels of total cholesterol and low-density lipoprotein cholesterol than the 482 gly(G) carriers. The 394-482G-528A haplotype was associated with protection from diabetes, while the 394A-482A-528A was associated with the diabetes.

Conclusions The results suggested that the 482Ser variant of PGC-1 α conferred the susceptibility to type II diabetes in south Indian population

J06.02

Kearns-Sayre syndrome and a mtDNA deletion in Bulgarian patient

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Kearns-Sayre syndrome (KSS) is a mitochondrial disease characterized by progressive external ophthalmoplegia, pigmentary retinitis and an onset before the age of 20 years. Common additional features include bilateral sensorineural deafness, cerebellar ataxia, heart block, skeletal muscle myopathy, intestinal, endocrine disorders and renal failure. Patients with KSS have a large deletion of mtDNA, ranging from 1,000 to 10,000 nucleotides. The condition is rare and most cases are sporadic. The risk of the mother being carrier of a large mtDNA deletion and transmitting it to her child has been estimated to be less than 4%.

Here we report a male patient with KSS. The first clinical findings dated from the age of 8 years and include bilateral ptosis, ophtalmoplegia. Around the age of 10 years the patient muscle weakness, dysmetria in climbing stairs and uprising, ataxic gait and tremor. The affected patient also showed positive test of Romberg and reflex of Babinski.

The molecular genetic testing by MLPA (SALSA MLPA P125-B1 mitochondria) revealed large deletion encompassing a number of mitochondrial genes *COX1*, *COX2*, *ATP6*, *COX3*, *ND3*, *ND4*, *ND5* (the location of the deleted probes along the mitochondrial genome 7146 - 13168). The deletion covers the genes involved in the oxidative phosphorylation pathway. The deletion is heteroplasmic ~ 50%.

We detected a classical heteroplasmic mtDNA deletion associated with KSS, but atypical clinical picture. Our patient did not show pigmentary retinitis, MRI showed normal bilateral optic nerve. No sensorineural impairment is detected. Our results showed that there is no simple genotype/phenotype correlation in KSS.

J06.03

Identification of a Novel N-acetylgalactosamine-6-sulfate sulfatase Gene Mutation in an Iranian twin with Mucopolysaccharidosis Type-IVA

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Background:mucopolysaccharidosis Type IVA or Morquio A Syndrome, which is inherited as an autosomal recessive trait, is a lysosomal storage disorder and results from the deficiency of N-acetylgalactosamine-6-sulfate sulfatase activity. This deficiency leads to lysosomal accumulation of keratin sulfate and chondroitin 6-sulfate. In this study, GALNS mutation analysis was performed on Iranian twin patients.

Methods: Mutation screening of the GALNS gene was performed by PCR and direct sequence analyses using genomic DNAsamples.

Results: Sequencing analysis revealed a novel homozygous missense mutation in theGALNS gene at c.148G>A [p. G50R] in Iranian twin MPS-IVA Patients.

Conclusion:this data may be useful for carrier detection and prenatal diagnosis in informative families whose specific mutations have not been identified.

J06.04

The clinical and lab manifestations of the onset of Methylmalonic aciduria

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Introduction: Methylmalonic aciduria (MMA) comprises a group of diverse inherited disorders causing methylmalonic acid (MMAc) accumulation in body fluids. The total incidence estimates to 1:48,000-100,000 newborns.

Material: We report on 5 Moldavian children diagnosed with MMA (3 newborns, 7 and 30 mo old). Amino acids by liquid chromatography (blood/urine), NMR spectroscopy (urine for organic acids) and tandem MS/MS were used for diagnosis.

Results: In three cases the clinical manifestations occurred in the neonatal period by: body weight loss [-15%], dehydration, generalized hypotonia, recurrent vomiting, seizures, frequent apnoea, precoma, severe metabolic acidosis: anion gap[17-26mmol/L], low pH[7.23-7.33], hyperlactacidaemia[2.6-8.0mmol/L], hypoglycaemia[1.8-2.8mmol/L], hyperammonaemia[126-390µmol/L], ketonuria. In one case clinical signs debuted in the first week of life, but not being diagnosed he continued to present frequent precoma, ketoacidosis and sings of pancreatitis being defined as a MMA at 30 mo old. In another case, patient presented late onset from 7 mo old associated with similar symptoms as the previous case. In all cases there were identified: high level of MMAc in urine, positive C3[6.49-21.39,ref.val<4.4] and C3/C2[0.33-1.05,ref.val.<0.2]. In four children with neonatal clinical onset the urine MMAc was over 19 mol/molCrea when diagnosed with severe clinical evolution, while in the late onset child the urine MMAc was under 2 mol/molCrea with mild evolution. There was no apparent correlation between

urine MMAc and C3. All children required a special diet.
Conclusion: The results support the need to develop a system for early diagnosis of IEM in Moldova. The neonatal onset is predictive for severe evolution of disease.

J06.05

A novel mutation of GALNS gene in mucopolysaccharidosis type 4A

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Mucopolysaccharidosis type IVA is an autosomal recessive lysosomal storage disease characterized by intracellular accumulation of keratan sulfate and chondroitin-6-sulfate. Key clinical features include short stature, skeletal dysplasia, dental anomalies, and corneal clouding. Intelligence is normal and there is no direct central nervous system involvement, although the skeletal changes may result in neurologic complications. There is variable severity, but patients with the severe phenotype usually do not survive past the second or third decade of life. N-acetylgalactosamine 6-sulfatase (GALNS) enzyme activity is recommended as the first diagnostic test, especially when clinical findings strongly indicate MPS IVA and urine GAG analysis is normal. GALNS enzyme activity can be measured in cultured fibroblasts or leukocytes.
Case Report: The case was a 12 years old boy born from consanguineous marriage due to normal pregnancy and delivery. He had normal growth and development until 2 years old. Then appeard spinal muscle weakness leading to kyphoscoliosis. The other clinical manifestations were ankle valgum, genu valgum, hip dysplasia and spinal cord compression that was operated 4 years ago.Molecular Genetic Testing: DNA sample of the proband was investigated for the mutation in coding exons of GALNS gene, and then the findings were compared with parents. A homozygous mutation at codon 181 TAC>TGC (Y>C) was found. The parents were heterozygous at this position. This mutation was not been reported yet. The result was analyzed by online PolyPhen-2 software too. Thus, this mutation could be considered as a pathogenic mutation for inducing the disease.

J06.06

Effect of sevoflurane anaesthesia on serum glutathione S-transferase concentration and the association with the CYP2E1, GSTA1 and GSTP1 genetic variants.

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Introduction: The serum glutathione S-transferase alpha (α-GST) concentration has been discovered as a marker of hepatocellular integrity after sevoflurane anaesthesia and mild impairment was observed. The role may play genetic polymorphism of genes, coding for enzymes involved in the metabolism and detoxification process o sevoflurane - CYP2E1, GSTA1 and GSTP1. GSTA1*B (-567T>G, -69C>T, -52G>A) allele is linked with reduced promotor activity, GSTP1*A, *B, *C are characterized by two amino acid substitutions Ile105Val (c.313A>G) and Ala114Val (c.341C>T), which also decrease the enzyme activity, while the variant -1053C>T in CYP2E1 gene is responsible for increased enzyme activity.
Materials and methods: 86 unrelated Polish patients undergoing sevoflurane general anaesthesia, were enrolled into the study. Measurement of serum α-GST concentrations in three time points: before anaesthesia, directly after and 24 hours after the end of anaesthesia was performed using ELISA. Molecular analyzes included sequencing of GSTA1 gene fragments and pyrosequencing for CYP2E1 and GSTP1 changes genotyping.
Results: The increase of α-GST concentration at the end of anaesthesia was observed in 54.7% of patients and 24 h later in 31.4% of subjects, but in all cases the increase was not statistically significant. Frequency of GSTA1*B was 43%, whereas the GSTP1 gene alleles 313G, 341T were found in 28% and 10% respectively. The CYP2E1-1053T variant was observed with 1% frequency. None of analyzed SNPs were significantly associated with the enzyme level.
Conclusions: We not observed any significant hepatotoxic effect in Polish patients after sevoflurane anaesthesia and the α-GST enzyme level was not associated with analyzed variants.

J06.07

A rare case of TMAU associated with suspected Currarino triad

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Trimethylaminuria (TMAuria; MIM 602079) is a metabolic disorder caused by failed N-oxygenation of the odorous compound trimethylamine (TMA) due to FMO3 gene dysfunctions. Several polymorphic variants of *FMO3* have been identified and, individually, these have little or no effect on enzyme activity. However, the haplotype with two polymorphisms in cis seems to cause a reduction of enzyme activity. Non-oxygenated TMA is a very strong-smell substance that is, than, expelled through urine, breath and sweat. The result is a severe body odor and associated psychosocial condition (1). Currarino triad (MIM 176450) is a multiple congenital anomalies syndrome characterized by three distinct clinical aspects: anal atresia, sacral anomalies and presacral mass. Genetic causes are mutations at *HLXB9*, a Homeobox gene encoding for a sequence-specific DNA binding protein implicated in the control of gene expression in both developing and adult tissues (2). Here we present a case of a 64 years-old woman that shows a severe fish-like body odor and all the malformations peculiar of Currarino syndrome. We described the first case in literature of TMAU syndrome in association with Currarino triad.
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J06.08

CTLA-4and PTPN22 genes expression as risk factorin egyptisn patient with typr 1diabeted

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Diabetes mellitus type 1 or T1DM; form of diabetes that results from the autoimmune Insulin action is the consequence „The CTLA4 gene is found on chromosome 2q33. Cytotoxic T-lymphocyte-associated protein 4 (CTLA4) is expressed only on activated T lymphocytes, and downregulates T cell function, limiting both activation and expansion. CTLA-4 is a surface molecule found on activated T cells which produces a negative signal by inhibiting the T cell receptor signaling interactions (blocks binding of CD80 and CD86). It is thought that inherited changes in CTLA-4 gene expression can increase T cell self-reactivity and therefore play an important role in autoimmune diseases such as type 1 diabetes(Protein tyrosine phosphatase, non-receptor type 22): PTPN22 The single nucleotide polymorphism located in the PTPN22 gene has been associated with autoimmune disorders, including an increased risk of Type 1 DiabetesThis work aims to report the prevalence and frequency of CTLA4 and PTPN22 genes expression as a risk factor in patients with Type-1 diabetes by Real-Time PCR and correlate the genes expression to each other anopportunities for early testing and diagnosis, .This study will include 100 patients with Type-1 Diabetes divided into two groups: 50 patients having Diabetes type-1 (Controlled) and 50 patients having Diabetes type-1 (insulin resistance); Together with 50 healthy age and sex matched controls.
These patients will be subjected to the following: Full Clinical evaluation Complete blood count and routine lab tests (FBS, PPBS, HbA1C and the Glucose curve).>3. Genetic analysis includes: Five (5) ml of blood on EDTA will be withdrawn, RNA extraction and quantitative assessment of CTLA4 and PTPN22 .

J06.09

Association analysis of BDNF gene Val66Met polymorphism and obesity: Case-control study

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Introduction: The brain-derived neurotrophic factor (BDNF) is one of the candidate genes for obesity. There is evidence that Val66Met polymorphism may be involved in the pathophysiology of obesity. In the present study, we investigated the relationships between this polymorphism and obesity and obesity-related clinical phenotypes in a population from Turkey.
Materials and Methods: A functional Val66Met polymorphism of BDNF gene was studied in patients with obesity (n=88) and non-obese controls (n=80). Genotyping of the Val66Met polymorphism of BDNF was performed by po-

lymerase chain reaction amplification and restriction fragment length polymorphism.
Results: The data showed that the BDNF Val66Met genotypes and their allele distributions did not differ between patients with obesity and the controls ($\chi 2$=0.06, df=2, p=0.806 and p=0.109, df=1, respectively). We also investigated the association between the BDNF Val66Met polymorphism and obesity-related clinical phenotypes; however, no significant association was observed.
Conclusions: Our data do not provide evidence that the BDNF Val66Met polymorphism may be involved in the etiology of obesity in a population from Turkey. So,our results do not support a significant role for the BDNF Val66Met polymorphism in the development of obesity in the population studied. The results of the present study suggest that the BDNF gene Val66-Met polymorphism do not play major roles in conferring susceptibility to obesity in a western population from Turkey. However, further studies with larger sample sizes assessing the associations between the BDNF gene polymorphisms and obesity should be performed in several other ethnic populations.

J06.10
Phenylketonuria frequency and PAH gene spectrum mutations in Rostov Region

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Phenylketonuria (PKU, MIM#261600) is an autosomal recessive hereditary disease referred to a group of enzyme defects. PKU frequency and PAH gene spectrum mutations varies between populations. Aim: To investigate PKU clinical and genetics characteristics in patients from Rostov Region (RR). Methods: Epidemiological, PCR, sequencing and MLPA.
Results: Within a more general framework of genetic and epidemiology study of RR population PAH gene mutations frequencies and PAH gene spectrum mutations for Russians in RR. There are 221 PKU patients in a RR Hereditary Diseases Register. PKU frequency in RR according to the data of the neonatal screening is estimated as 1:4978. Russians are the most substantial ethnic group composing a patients' sample (90.05%). Comprehensive DNA diagnostics detect PAH mutations in Russian PKU patients (90 people, 180 chromosomes). 27 different mutations represent the spectrum, 9 mutations are frequent. The most prevalent is R408W (71.11%), second-frequent - IVS12+1G>A mutation (5%), it is followed by P281L and R158Q (2.2% cases for each mutation). The R252W mutation occurs in 1.67% cases, IVS4+5G>T, IVS10-3C>T and EX5DEL - in 1.1%.cases for each mutation. A300S and R297H are revealed in two patients from one family with a mild form of PKU. The rest 16 mutations (A342T, A403V, E280K, F39del, F299C, IVS2+13T>G, IVS7+1G>A, IVS9+5G>A, IVS10-11G>A, K363fsdelG, R176X, R408Q, Y268C, p.N133_Q134>Rfs, c.47_48delCT, p.V245A) are detected in single cases (8.89% in total). PAH gene mutant alleles diversity is revealed in population of one ethnic affiliation (people, who class themselves to Russians), PAH mutations frequency profiles might vary considerably. The study was partially funded by the RFBR grant 14-04-00525.

J06.11
Cystic fibrosis related bone disease

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Cystic fibrosis (CF) is the most frequent monogenic genetic disease, autosomal recessive transmitted, characterized by important clinical polymorphism and significant lethal prospective. CF related bone disease occur frequently in adults with CF and the childhood is the period of bone formation, children being more susceptible to low bone density. Several factors like pancreatic insufficiency, hormone imbalance and physical inactivity contribute to CF bone disease development and their revealing would be important for prophylactic attitude against bone disease occurrence.The study was observational, transversal, with a cross-sectional design.
Objective: Evaluation of CF bone disease presence and identification of its risk factors in our CF children population. Subjects and methods: Study included 68 children with cystic fibrosis, genotyped and monitored in the National CF Centre. At the annual assessment, besides clinical examination, biochemical evaluation for pancreatic insufficiency, diabetes, a subgroup of 26 children were evaluated for bone mineral density using dual energy x-ray absorptiometry (DXA).
Results: Twenty-six patients, aged over 10 years were diagnosed with CF bone disease, without significant gender gap. Bone disease was frequent in patients aged over 10 years with exocrine pancreatic insufficiency, carriers of severe mutations and CF liver disease.
Conclusion: CF children carriers of a severe genotype who associates pancreatic insufficiency and CF liver disease were more likely predisposed to low bone mineral density. Further studies should discover other significant influences in order to prevent the development of CF bone disease and an improved life quality in CF children.

J06.12
Cystic fibrosis and Clostridium

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Background:Children with cystic fibrosis are, unfortunately, candidates at multiple antibiotic courses,having a potential increaserisk for pseudomembranous colitis with Clostridium difficile. The aim of the paper was to evaluate the frequency of Clostridium difficile infection among children with cystic fibrosis(CF).
Methods: Retrospective analysis over a ten years period was done, using the information from our CF center`s database. In all the patients, only presentation with diarrhea occurred during antibiotherapy were taken into consideration. Diagnosis test for Clostridium difficile infection was performed by enzyme immunoassay for detection of toxins A and B.
Results: Over a ten years period, 308 patients with cystic fibrosis were admitted in our clinic; only five of them(1.62%) were diagnosed with Clostridium difficile infection. Patients were diagnosed in the last 4 years, by the detection e of toxin A or toxin A and B(in 2 patients) in the presence of diarrhea; they had a favorable outcome, with a good response to treatment(metronidazole in 3 cases, metronidazole and vancomycine in 2 cases). All patients had chronic Pseudomonas aeruginosa infection and received more than fourteen days of antibiotics.
Conclusion: Clostridium difficile infection should be consider for evaluation in cystic fibrosis patients with diarrhea who receive antibiotics. Special attention is necessary when antibiotherapy is given for a long time, as commonly reccomended in cystic fibrosis patients.

J06.13
Familial intrahepatic cholestasis: new approaches to diagnosis

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***Introduction*:** Familial intrahepatic cholestases (FICs) are a heterogeneous group of autosomal recessive disorders of childhood with cholestasis of hepatocellular origin. Three distinct forms are described: FIC1, FIC2 and FIC3 due to mutations in *ATP8B1*, *ABCB11* and *ABCB4*, respectively. Different mutations in these genes may cause either a "malignant" progressive familial intrahepatic cholestasis (PFIC) or a benign recurrent intrahepatic cholestasis (BRIC). Liver histology is important, but not specific, for diagnosis. Genotyping is conclusive. To exclude rare forms of transmission, the study of parents is required and to sort out the meaning of the novel mutations a molecular study of healthy controls and protein modelling are mandatory
Methods: For the purposes of the present study we genotyped 27 children with intrahepatic cholestasis, diagnosed on either a clinical or histological basis.
Results: Three BRIC, 22 PFIC and 2 BRIC/PFIC were identified. Thirty-four different mutations were found of which 16 were novel. One was a 2Mb deletion (5'UTR- exon 18) in *ATP8B1*. In another case microsatellite analysis of chromosome 2, including *ABCB11*, showed uniparental disomy. Two cases were compound heterozygous for BRIC/PFIC2 mutations. The occurrence of mutations in the same genes in PFIC and BRIC indicates a *continuum* in severity of the involved mutations, mostly exemplified by intermediate BRIC/PFIC form.
Conclusion: Our results highlight the importance of the pathogenic role of

novel mutations in the three genes, the unusual modes of their transmission and the recognition of an intermediate BRIC/PFIC form as a specific entity.

J06.14
Are we ready to translate therapies targeting mitochondrial dysfunction to people with Down syndrome?

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Mitochondrial dysfunction is consistently observed in Down syndrome (DS). It possibly contributes to determine DS mental retardation as well as other phenotypic abnormalities including Alzheimer's disease, type2 diabetes, obesity, and hypertrophic cardiopathy. In human DS cells we demonstrated that mitochondrial dysfunction is associated to the downregulation of nuclear encoded mitochondrial genes (NEMGs). We identified the nuclear receptor interacting protein 1 (NRIP1/RIP140), a gene mapping to the chromosome 21 and overexpressed in DS cells, as a good candidate for NEMG downregulation. We further demonstrated that NRIP1 transient attenuation is able to counteract mitochondrial dysfunction in DS fibroblasts. NRIP1 is known to affect oxidative metabolism and mitochondrial biogenesis by negatively controlling mitochondrial pathways regulated by PGC-1α.
Based on this background, we are pursuing a project to correct mitochondrial alterations in DS by pharmacologically modulating the activity of the NRIP1 targets PGC-1α and PPARs. To this aim we have supplemented cultures of fibroblasts from DS human fetuses with drugs affecting respectively PGC-1α and PPAR activity, namely metformin and pioglitazone, to evaluate their modulatory impact on mitochondrial function and morphology. We found this strategy effective on rescuing mitochondrial function in terms of oxygen consumption, ATP production and mitochondrial biogenesis.
Counteracting mitochondrial dysfunction in DS will open new therapeutic perspectives to improve the DS neurological phenotype and to prevent DS associated pathologies. The results of this study may be easily extended to other neurodegenerative disorders associated to mitochondrial dysfunction.

J06.15
Mutation spectrum of phenilketonuria in Karachay-Cherkessia

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The prevalence of phenylketonuria in Russia is 1:8000-10000 infants. The prevalence of phenylketonuria in Karachay-Cherkessia is about 1: 3000. We have compiled and analyzed the results of a comprehensive population and medical genetic studies of ten districts and two cities in Karachay-Cherkess Republic, and found 36 unrelated patients with phenylketonuria.
All patiens were assayed for the presence of mutations in the PAH gene by the following algorithm. Initially, we searched for 8 frequent mutations by MLPA-analysis. This system has an efficiency up to 81% for Russian patients. The most common R408W mutation in the PAH gene for the Europeans met 3 times (4.2%), mutations R158Q and IVS10-11G> A met once (1.4%). It was further used MLPA-analisis of 10 recurring mutations. R261X mutation met at 50 chromosomes (69.4%), Y414C and L48S met once (1.4%). The detections of the second mutation in some patients was conducted using Sanger sequencing of the PAH gene. R413P mutation was found on 6 chromosomes (8,3%), P211T on 3 chromosomes (4,2%), F331S on 2 chromosomes (2.8%), mutations P211L, S349P, s.664_665delGA, c.1089delG met once (1.4%).
Mutations frequent for the Europeans were found only in Russians by nationality. For indigenous people of the republic - Karachai, Circassians, Abasins - frequent mutations are R261X, R413P, P211T, F331S, which according to the results of our study have met more than once. Widespread of R261X mutation can be explained by a high inbred of Karachay-Cherkessia population, and probably a founder effect.
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J06.17
Expression analysis of genes related to inflammation in patients with Gout disease

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Introduction: Gout is a multifactorial disease characterized by recurrent attacks of acute inflammatory arthritis. It is characterized by elevated levels of uric acid in the blood leading to deposition of uric acid crystals in joints, tendons and surrounding tissues. This condition increases the risk of hypertension, diabetes, metabolic syndrome, renal and cardiovascular disease. The Gout become more common in recent decades, affecting more than 2% of the populations.
Materials and methods: We have performed an expression analysis of 34 Bulgarian male patients with Gout disease and 34 healthy controls. RNA was isolated from blood samples and Real Time PCR analysis using Custom RT2 Profiler PCR Array kit, containing 11 genes (NOD2, NLRP3, PYCARD, IL1B, MYD88, P2RX7, NFKB1, CASP1, CASP5, TRAF1, IRAK1) was applied.
Results: The obtained results showed an increased expression of genes NOD2 and IL1B. The Nod2 is cytosolic receptor and its role is related to modulate the cytokine production by Th1 cells. The Il-1β is an important mediator of the inflammatory response and is involved in cell proliferation, differentiation and apoptosis. The Il-1β predominantly derived from the innate immune cells and Th1 cells. The higher expression of these genes is connected to the acute inflammatory reaction.
Conclusion: New data about the role of NOD2 and IL1B in the pathophysiology of the Gout disease have been received.

J06.18
Sequence Analysis of MT-ND Genes from Iranian Patients with Leber's Hereditary Optic Neuropathy

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Background. Leber's hereditary optic neuropathy (LHON) is an optic nerve dysfunction due to mutations in mitochondrial DNA (mtDNA) which is transmitted in a maternal pattern. It is caused by three primary point mutations, G11778A, G3460A and T14484C, in the mitochondrial genome. These mutations are sufficient to induce the disease, accounting for the majority of LHON cases, and affect genes that encode for different subunits of mitochondrial complexes I and III of the mitochondrial respiratory chain. Other mutations, called secondary mutations, are ordinarily associated with primary mutations. There is currently no effective treatment for LHON.
The purpose of this study was to determine MT-ND variations in Iranian patients with LHON.
Methods. In order to determine the prevalence and distribution of mitochondrial mutations in the LHON, the mtDNA was studied through PCR amplification and DNA sequence analysis.
Results. In 35 LHON patients, MT-ND gene sequencing revealed a total of 44 nucleotide variations (30 types) in the twenty-seven LHON patients, but eight patients had no changes in the ND genes. Of the 30 types of changes, 50% were previously reported and 50% were novel changes.
Conclusion. This clustering of mutations is therefore useful to extend the mtDNA polymorphism database of Iranian patients and should facilitate definition of disease-related mutations in human mtDNA. This research may help to understand the disease mechanism and may open up new experimental and therapeutic opportunities of LHON for diagnostic testing and for future investigations.

J06.19
The prevalence of Melanocortin-4 receptor gene mutations in Slovak obese children and adolescents

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Introduction: Melanocortin-4 receptor (MC4R) deficiency is the most frequent monogenic form of obesity. The contribution of MC4R mutations on Slovak population has not been investigated as yet.
Material and methods: 210 unrelated Slovak obese patients were analysed. Body weight and height were recorded for this cohort group, SDS BMI was counted and subsequent standard biochemical protocols assessed their glucose, triglycerides, total cholesterol and lipids blood levels. The coding region of MC4R gene was screened by sequencing analysis. Multifactorial statistical analysis was performed in order to determine the influence of genotypes on standard biochemical blood markers.

Results: Four different mutations in four patients were identified giving a detection rate of 1.9%. Of these, three were missense mutations previously identified and characterized by other research groups (p.R7C, p.S127L and p. R305W, respectively). One was a novel nonsense mutation p.W174* detected in a severely obese 7 years old boy. This mutation was further analysed in family segregation analysis and exhibited variable penetrance. Two known amino acid polymorphisms (p.V103I and p.I251L) were also identified in 7 subjects of our cohort group. No significant influence was observed in carriers of DNA variants on tested parameters.
Conclusions: MC4R mutations in Slovak obese children and adolescent seem to appear with low incidence of 1.9%. We identified three missense mutations and one novel nonsense mutation, which was further studied in family segregation analysis. Our findings support variable penetrance of the MC4R gene variations and also agree with age-dependent manifestation manner. Not all of the mutation carriers fulfilled the typical symptoms of MC4R deficiency.
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J06.20
The gene expression of SIRT1 ,SIRT3 and SIRT4 in patients with type 2 diabetes mellitus: The probable association with its related complications

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Introduction: Sirtuins are NAD +-dependent deacetylases that have been linked to cellular energy metabolism, survival and the redox state. Among them, SIRT1, SIRT3 and SIRT4 have important roles in this regard. Since dysregulation of energy metabolism, oxidative stress and aging have been implicated in the pathophysiology of diabetes and its associated complications; we aimed to measure SIRT1, SIRT3 and SIRT4 mRNA levels in patients with type 2 diabetes based on either with or without retinopathy and /or nephropathy.
Methods: A total of 38 patients with type 2 diabetes were included in the study. The participants were categorized into two separate groups: Patients with retinopathy and/or nephropathy (group1) (n=19) and patients without retinopathy and/or nephropathy (group2) (n=19). We compared mRNA levels of SIRT1, SIRT3 and SIRT4 in PBMCs isolated from participating subjects using real time RT-PCR.
Results: The patients in two group were matched in term of age (p=0.09), sex (p=0.1) and BMI (p=0.08),whereas the duration of diabetes was significantly (p=0.004) higher in group1 (16.37± 5.6 years)compared with group2 (9.9±7.1 years). We found a significant (p=0.001) increase in relative SIRT1 gene expression in group 1 in comparison with the group 2, while SIRT4 mRNA levels were significantly (p=0.013) lower in group 1. In this study, there was no significant difference in SIRT3 mRNA levels between two groups.
Conclusions: It seems that the relative expression of SIRT1 and SIRT4 may be involved in an etiological process that leads to diabetes associated complications.

J06.21
Clinical and molecular characteristic of X-linked adrenoleukodystrophy, reporting of 9 new cases including one novel mutation from Iran

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X-linked adrenoleukodystrophy (X-ALD) is a rare peroxisomal disorder resulting in progressive cerebral demyelination, axonal dysfunction, and adrenal insufficiency. It is the most common peroxisomal disorder with an estimated birth incidence of about 1 out of every 20,000. There is no ethnic predominance.
The age of onset and morbidity are highly variable and progression is unpredictable. Male hemizygotes may initially present with neurological symptoms in two different forms: (X-ALD) with childhood presentations, and Adrenomyeloneuropathy (AMN) that presents in adulthood.
Primary manifestations of X-ALD are moderate cognitive deficits followed by diminished visual acuity, central deafness, cerebellar ataxia, hemiplegia, convulsions and dementia leading to a neurovegetative state or death within mostly in two years.
X-ALD is inherited in an X-linked manner and mainly affects 4 to 8 year old boys. About 93 to 95% of index cases have inherited the ABCD1 mutation from their carrier mothers, and 5 to 7% of individuals have a de novo mutation. ABCD1 is the only gene known to be associated with this entity.
The diagnosis of X-ALD is based on clinical findings. MRI is always abnormal in males with neurologic symptoms and often provides the first diagnostic clue. Plasma concentration of very long chain fatty acids (VLCFA) is abnormal in 99% of males with X-ALD.
Here we report 8 affected boys with X-ALD from Iran. VLCFA was increased strongly, and MRI images were typical in all of them. Molecular analysis of ABCD1 gene confirmed the diagnosis in 4 of the patients and we detected 1 novel mutation in an index case.

J07.01
Recombinant Antigen

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Examine to evaluate serological applications of *Toxoplasma gondii* rhoptry protein1 antigen
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Abstract
Objective: Toxoplasma gondii is distributed across a worldwide and infects most species of warm-blooded animals, including humans. The heavy incidence and severe or lethal damage caused by *T. gondii* infection clearly indicates the need for the development of a vaccine. The current study goals were to evaluate serological applications of *Toxoplasma gondii*rhoptry protein 1 (ROP1) antigen.
Materials and methods: We constructed DNA vaccines by using the eukaryotic plasmid pROP1. Purification by one-step metal affinity chromatography allowed recovery of milligram amounts of pure recombinant proteins per liter of culture. The usefulness of this antigen for diagnosis of human infections was provided and tested on 77 serum samples which are obtained during routine diagnostic tests.
Results: A panel of 20 serum samples from patients with acute toxoplasmosis was compared to a panel of 35 serum samples from individuals with chronic toxoplasmosis. The results indicated that ROP1 recombinant antigen detected antibodies more frequently in samples from individuals with acute infections (96%) than in samples from individuals with chronic infections (17%). These results suggest that an immunoglobulin G antibody against ROP1 antigen is produced during the acute stage of toxoplasmosis but are uncommon in the chronic phase of the infection.
Conclusion: Hence, this recombinant protein can be used as specific molecular markers to differentiate between acute and chronic infections
.
Key words:Toxoplasma gondii, rop1, recombinant antigen

J07.02
Antibiotic resistance

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The prevalence of Antibiotic resistance and corresponding resistance genes in clinical isolates of staphylococci from Kurdistan hospitals
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Background and Objectives: Staphylococcus aureus is one of important etiology of contagious infections in hospital. Aminoglycosides still play an important role in anti staphylococcal therapies, although emerging resistance amongst staphylococci is widespread. This study was conducted to further our understanding of aminoglycoside resistance in Kurdistan hospitals of Iran.
Materials and Methods: we tested 212 clinical isolates of *Staphylococcus aureus* for susceptibility to penicillin, erythromycin, clindamycin, tetracycline, vancomycin and methicillin. All of these isolates were screened for the presence *ermA ermB ermC mphC msrA, van A, tet K, tet M, tet L, tet O* and *mecA* genes.
Results: The highest rates were seen in treatment with *penicillin (93%), Methicillin (77/33%), tetracycline (61/33%), clindamycin (58/6%), erythromycin (54/6%)* while the lowest sensitivity was observed in *vancomycin(12%)*.

Foremore, The most prevalent gene was *mecA* followed by *ermA*, *tetK* , *ermC*, ermB, tetM,msrA, mphC, *tetL* and *tetO* and *van(A)* didn't see in any isolated.
Conclusion: This study indicates that resistance to erythromycin and tetracyclines are mainly by efflux pumps mediated by *ermA* ,*ermC* and *tetK* genes in S. aureus in Kurdistan province, respectively. Our results also revealed the need for further investigations using a higher number of specimens representing a wider variety of locations to determine the antibiotic resistance patterns in our state more precisely.
Keywords: Staphylococcus aureus, Antibiotic resistance, Kurdistan

J07.03
Cytokines genotypes as predictors of disease outcomes in HIV-1 infected Ukrainians

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Background. Cytokines genes single nucleotide polymorphisms (SNPs) influence clinical course of HIV infection in different population groups. The objective of the research was to determine cytokines genotypes association with outcomes of the disease in Ukrainians with HIV-1.
Methods. We examined promoter SNPs in IL-4 (rs 2243250), IL-10 (rs 1800872), TNF-α (rs 1800629) among 78 naive HIV-1 infected European Ukrainians (68 % male, 32 % female; age at diagnosis (33,35±0,76) years) and 100 healthy controls using PCR-RFLP. Patients with HIV-1 were distributed into groups depending on implications of bacterial, viral, fungal and parasitic infections.
Results. All detected cytokines SNPs showed no significant deviation from the Hardy-Weinberg equilibrium in controls (p≥0.1). The dissimilarity in cytokines genotypes frequencies among HIV-1 infected persons with different clinical course of the disease was determined. We found the association of C/A IL-10 with bacterial (OR=0.74, p≤0.05), C/C IL-10 and G/A TNF-α - viral (OR=0.65, p≤0.05; OR=0.53, p≤0.05 appropriately), C/T IL-4 - fungal (OR=0.44, p≤0.05) infections. Cytokines genes variants played a protective role in patients with HIV-1: A/A IL-10 genotype in fungal (OR=3.98, p≤0.05), C/A IL-10 in viral (OR=1.93, p≤0.05), G/G TNF-α in viral and parasitic opportunistic infections (OR=1.83, p≤0.05, OR=1.79, p≤0.05 appropriately).
Conclusions. Our results demonstrate that cytokines genes polymorphisms may be used as clinical markers to predict outcomes of HIV-1 infection and warrant further studies in the host genetic factors sphere.

J07.04
Trials for Defining the Mutation Spectrum in Egyptian FA patients

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Introduction: FA-A is the most frequent complementation group representing approximately two-thirds of the patients in the majority of countries.
Aim of the work: screening the most internationally reported mutations of FANCA gene in the Egyptian (FA) Fanconi anemia patients and evaluate a correlation between clinical and cytogenetic results.
Subjects and Methods: Fourty five cases, descending from unrelated consanguineous pedigrees were recruited from the hereditary blood disorders (HBD) clinic, NRC. All patients had positive chromosomal breakage studies with diepoxybutane (DEB) confirming the diagnosis of FA. Amplification of FANCA gene exons of interest by PCR, Multiplex PCR, Restriction Analysis, DNA sequencing for patients and controls was done.
Results: 13 % (6/45) of our patients had homozygous deletion of exons (12-31) of the FANCA gene, these patients presented with severe phenotype, 70% were born with low birth weight. All patients had cafe´ au lait spots, hyper pigmentation, and 86% suffered skeletal abnormalities. Increased chromosomal breakages by DEB with an average of 12.5 break/cell were detected among those patients. No 2574C to G (S858R) mutation in exon 27or c.3788_3790 del TCT mutation in exon 38 in the FANCA gene was detected in our studied patients. No new mutations were detected in exons 34 and 43 of the FANCA gene. **Conclusion**: This is the first step in defining the mutation spectrum of FA. 13 % (6/45) of our patients had homozygous deletion of exons (12-31) of the FANCA gene, these patients presented with severe phenotype. Further studies on a larger number of patients are recommended to be able to define Egyptian FA mutations' spectrum.

J07.05
Investigation of IL-12p40, IL-12Rβ1, IFNγR1, IFNγR2 gene mutations in childhood tuberculosis

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Background: Tuberculosis is a necrotizing, chronic infection caused by a group of mycobacteria known as Mycobacterium tuberculosis complex (MTC). About 9.0 million newly diagnosed tuberculosis cases and 1.5 million tuberculosis deaths are estimated to occur worldwide in 2013. Mycobacterium Tuberculosis is responsible for almost 99% of the cases. Clinically apparent infections occur in only 5 to 10% of all cases. Ethnic differences, twin studies, animal models and family segregation analyses support the hypothesis of genetic susceptibility to tuberculosis. Mendelian susceptibility to mycobacterial diseases (MSMD) is a rare congenital disease, characterized by hereditary immunodeficiency causing mycobacterial infections. Mutations in IFN-γ-IL-12 pathway are considered to play a role in MSMD. Identifying MSMD mutations may contribute to explore molecular mechanisms in host's immune response. Aim: To investigate the role of mutations in IFNGR1, IFNGR2, IL12B and IL12RB1 genes for developing MSMD in pediatric patients with extrapulmonary tuberculosis
Methods: Mutations were investigated in IFNGR1, IFNGR2, IL12B and IL12RB1 genes by sequencing in 30 pediatric patients with extrapulmonary tuberculosis.
Results: No mutation was detected in four genes which are involved in IFN-IL12 pathway. However, we found polymorphisms which might cause susceptibility to tuberculosis: rs7749390 in IFNGR1 gene , rs9808753 in IFNGR2 gene, rs11575926 in gene IL112B , rs3212227 and rs436857 polymorphism in IL12RB1 gene.
Conclusıon: In this study, we report genetic analysis of IFN-IL12 pathway on MSMD cases for the first time in the Aegean region of Turkey. Determining the frequencies of these polymorphisms in control samples and performing functional studies might elucidate the exact roles of these polymorphisms in TB infection in Turkish children.

J07.06
The impact of Xmn1-HBG2 , BCL11A, HBS1L-MYB, age and sex on HbF variation among Iranian hematologicaly normal individuals

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The impact of HbF on disease severity of Sickle cell and Beta thalassemia is well documented. Xmn1-HBG2, BCL11A and HBS1L-MYB SNPs have been introduced as the most important factors causing variation in HbF levels. However the impact of these factors on HbF variation could be population specific and depends on the frequency of the HbF boosting allele (minor allele) in each population. Determining the effect of these factors in Iran, where thalassemia is the most common genetic disorder, is important. In here we genotyped 8 SNPs including Xmn1-HBG2, 5'HS4, 2 BCL11A and 4 HBS1L-MYB SNPs in 122 Iranian hematologically normal individuals, including 51 male and 71 female. HbF values ranged from 0.3%-1.3% with a mean of 0.74 ± 0.21. Multivariate linear regression analysis was used to evaluate the association between HbF and these SNPs. No association was observed between these SNPs and HbF levels of the normal individuals. This study could suggest that these HbF modifying factors are not able to explain common HbF variations in the Iranian normal population. In support of this controversy, the ability of SNPs in these three loci, in explaining the total HbF variation has been considerably different between European healthy adults (44%) and African patients with SCA (16-20%). Therefore, to further study the major regulators of common HbF variation in normal population of Iran, studies with larger population sizes and more number of SNPs are proposed. This study was funded by the Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran.

J07.07
Mutation screening of the KLF1 gene by using SSCP in a cohort of Iranian B-thalassemia patients

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Krűppel-Like Factor 1(KLF1) is an essential erythroid-specific transcription factor. Mutations in the human KLF1gene have different phenotypic effects, ranging from increased HbF levels to the disruption of erythropoiesis. Here, we have screened 227 Iranian β-thalassemia (β-thal) patients for the presence of KLF1mutations by using the single-strand conformational polymorphism (SSCP) approach. Our aim was to assessthe potential effect of these mutations on the β-thal disease severity. After screening, two variants were found. One patient carried a potentially deleterious variant (Polyphen-2) in

exon 2 (p.F182L). Another patient was homozygous for a previously unreported intronic variant (c.911+84A>G). The patient with the p.F182L variant (c.544 T>C) had noticeably high HbA 2 levels (7.6%), consistent with the phenotypic effect of several previously characterized KLF1mutations in the same exonic region.
In addition,he had higher platelet counts(1069000/µl)compared to other patients in the cohort

J07.08
Genetic Predisposition In Aggressive Periodontitis Expressed By Interleukine-1 Gene Polymorphism

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Introduction: Aggressive periodontitis has a complex multifactorial etiology, genetic predisposition being one of the risc factors. Among the inflammatory mediators in aggressive periodontitis, interleukine-1, codified by IL-1A and IL-1B genes, has an important role in tissue destruction. The aim of this study is to identify if there is an association between interleukine-1 gene polymorphism with the aggressive periodontitis.
Materials and Methods: We comprised into our study 54 subjects (by clinically orodental examination): 17 healthy subjects and 37 patients with aggressive periodontitis. For the genetic polymorphism of IL-1 gene we used PCR reaction - GenoType IL-1 (HAIN Lifescience, Germany). It detects IL-1A-889 and IL-1B+3953 from epithelial cells on buccal mucous (collected using sterile swabs). Statistical analysis was made using ANOVA test and Pearson correlation coefficient.
Results: Regarding IL-1A-889 we found heterozygous (C/T) and homozygous (T/T) profile at 9 (52.9%) heathy subjects, who will require special monitoring for possible onset of aggressive periodontitis, and at 27 (72.9%) patients. We identified significant difference in the frequence of the positive genotype between the healthy subjects and patients: for C/T p=0.023 and for T/T p=0.0006. Also, IL-1B+3953 polymophism was found at 7 (41.2%) heathy subjects and 30 (81.1%) patients and we also identified a significant difference for C/T p=0.001 and for T/T p=0.0001 between the study groups.
Conclusions: Our study demonstrates that the IL-1 gene polymorphisms are associated with aggressive periodontitis. Single nucleotide polymorphisms at IL-1A-889 and IL-1B+3954 loci identified at healthy subject may be useful as risk markers in producing aggressive periodontitis.

J07.09
Molecular analysis of MEFV gene polymorphisms and mutations in rheumatoid arthritis in the Azeri population of Iran

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Objective: The aim of our study was analyzing the Mediterranean fever (MEFV) gene polymorphisms and mutations on exon 2 and 10 in patients with rheumatoid arthritis and to then compare disease activity between mutation carriers and non-carriers in the Azeri population of Iran.
Methods: In this cross sectional study we considered the MEFV gene polymorphisms and mutations in exons 2 and 10 in 50 Iranian Azeri patients with rheumatoid arthritis by polymerase chain reaction and direct sequencing.
Results: Thirty three out of 50 RA patients were found to carry MEFV polymorphisms and mutations. The most common were the D102D, G138G, and A165A polymorphisms. In comparison with the normal Azeri population the carrier state of MEFV mutations in our study patients was higher. No significant difference was seen in the disease activity between carriers and non-carriers.
Conclusion: MEFV mutations may act as a genetic susceptibility factor for RA. However, it has no major effect on the activity of disease in the Azeri population of Iran.

J07.10
The molecular basis of Thalassemia intermedia in central Iran

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Thalassemia intermedia is a clinical entity characterized by moderate, non-transfusional anemia and hepatosplenomegaly. To elucidate the mild phenotype of many patients with thalassaemia intermedia we screened three predominant mutations (IVSII.1 G:A, IVSI.110 G:A and IVSI.5 G:C mutations) in β-globin gene , deletion in α-globin genes, XmnI polymprphism and RFLP haplotypes at β-globin gene cluster in 50 thalassemia intermedia patient. 58% (29) of patients were associated with mentioned mutations. None of 29 patients parents had an elevated level of Hb F. IVSII-1 (G→A) mutation was linked to haplotype +-++ (57.69%). This haplotype is in linkage desequilibrium with Xmn –I polymorphism (C →T - 158) and has been associated with increased expression of HbF in thalassemia intermedia patients. Relatively mild clinical course was shown with homozygosity for this haplotype. Xmn I polymorphism was found in association with this prevalent mutation and was detected in the homozygous state in majority of the of the patients homozygous for the IVS-II-1 (G → A) mutation. Homozygoutes for Xmn-I (+) polymorphism have milder disease than heterozygotes. One patient had a single α-globin gene deletion (-$\alpha^{3.7}$ kb deletion). The main factor in mild phenotype thalassemia intermedia patienes was linkage of a XmnI(T) polymorphism with mutation IVSII-1 (80.76%),. which were associated with increased production of fetal hemoglobin and co-inheritance haplotype +-++ with thalassemia intermedia, especially with homozygous IVSII-I mutation Molecular basis of thalassemia intermedia as defined in this study explains the involvement of different factors that tend to develop the disease phenotype.

J07.11
Polymorphisms in the tumor necrosis factor receptor genes affect the expression levels of membrane-bound receptors

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Tumor necrosis factor (TNF) is a pro-inflammatory cytokine involved in a wide range of physiological processes. The biological activity of TNF is dependent on the expression level of its membrane-bound receptors. The aim of this study was to establish associations between single nucleotide polymorphisms (SNPs) in the TNF receptor genes (TNFRSF1A, TNFRSF1B) and membrane-bound TNF receptor expression levels on subpopulations of peripheral blood mononuclear cells (PBMC) and to determine the serum levels of soluble TNF receptors (sTNFRs) in healthy individuals and patients with multiple sclerosis (MS). Determining the expression level of membrane-bound TNF receptors on PBMCs was performed by flow cytometry using BD QuantiBRITE calibration particles. Serum levels of sTNFRs were determined via ELISA and genotyping was carried out using PCR-RFLP.
Individuals with genotype TT in SNP TNFRSF1A rs4149570 showed lower levels of sTNFR1 compared to GG genotype carriers. The genotype CC in SNP TNFRSF1B was associated with a lower percentage of CD14+ cells expressing TNFR2 compared to individuals with the CT genotype. The frequencies of alleles and genotypes of TNFRSF1A and TNFRSF1B SNPs had no statistically significant differences in MS patients and healthy individuals.
Polymorphisms in the TNF receptor genes represent one mechanism affecting the expression levels of membrane-bound receptors and TNF-mediated signaling.
The research was supported by „Scientific and Educational Foundation for Young Scientists of Republic of Sakha (Yakutia)“ (2014-01-0011).

J07.12
Cystic fibrosis and mannose binding lectine

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Background: Mannan-binding lectine (MBL) is a protein implicated in immune response in children. Children are more prone to develop pathologies like infection, autoimmune disease, cystic fibrosis, liver disease. The aim of the study was to evaluate the MBL serum levels in children with cystic fibrosis and liver disease, compared to controls. Material and methods: Patients with cystic fibrosis and liver disease were aged matched with controls, blood samples were collected. MBL was determined by solid-phase enzime-linked immunoabsorbent assay. Results: In the group of patients with liver disease associated with cystic fibrosis, the average was 2061.99 ng/ml , almost 50% lower than average level in the controls group (3986.827 ng.ml), being significantly low among children with cystic fibrosis and liver disease.

In our study MBL values ranged from 2.3 ng/ml to 5432 ng/ml. Conclusion: Determining of MBL levels in children is suggestive for certain diseases, like cystic fibrosis liver disease and could be used as a predictive factor for early diagnosis.

J07.13

The investigate of the biological association between atopic bronchial asthma and tuberculosis

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Atopic bronchial asthma (BA) and tuberculosis (TB) have become two of the most important global public health problems. Co-existence of BA and TB is extremely uncommon in the same individual (dystropic). We hypothesize that dystropic relationships between diseases are caused by shared proteins/genes that suspend simultaneous development of diseases.
Using system biology analysis we have determined of shared proteins/genes for BA and TB, assuming that the rare co-occurrence can be attributed to shared biological mechanisms.
Two associative protein networks of BA and TB were reconstructed using the ANDCell software. We found the 19 proteins associated with both BA and TB. Among the shared proteins identified in the study is gene *SPP1* previously not studied for TB, genes *CXCL10*, *TNFRSF1B* are not studied for BA, and genes *CD4*, *CD79A* are not studied for both BA and TB in "case-control" studies.
The identified shared proteins have been classified for Gene Ontologies using BiNGO plug-in of the Cytoscape platform. GO enrichment analysis for 19 shared proteins revealed 1262 statistically significant biological processes. Top 10 processes were related to immune response with "positive regulation of immune system process" being the most significant (p-value=$4.31 \bullet 10^{-16}$). The study has also identified shared genes related to crucial biological processes for life including of immune processes. One possible explanation for BA and TB appear to be inversely associated is that shared proteins operate mainly in area of immune processes which important for both diseases.
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J07.14

Results of molecular analysis of NLRP3, MVK and TNFRSF1A genes in patients with autoinflammatory diseases and juvenile arthritis

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Background: Human autoinflammatory diseases (HAIDS) are a group of rare heterogeneity genetic determined disorders caused by primary dysfunction of the innate immunity. More than 25 causative genes are known. The most common are NLRP3, TNFRSF1A and MVK. Systemic onset juvenile arthritis (soJA) has similar features so it's belonging to HAIDS is discussed.
Objectives: To discover contribution of mutations of NLRP3, TNFRSF1A and MVK to HAIDS in Russian patients. To define presence of the same defects in patients with soJA.
Material and methods: We recruited 40 patients with assumption of HAIDS and 39 pts with soJA. Selection criteria were persistent fever, maculopapular rash, arthralgia/arthritis, acute-phase markers. Diagnosis of soJA was based on ILAR criteria. DNA samples were extracted from whole blood and analyzed by direct automatic sequencing.
Results: Among HAIDS patients 9 cases were confirmed (22,5%). Six patients are heterozygous for mutations in NLRP3, 2 - in TNFRSF1A and 1 is compound heterozygote for mutations in MVK, included the new mutation c.1126G>C. Among arthritis pts 4 show genetic defects (10,3%): 2 of them are heterozygous for mutations in NLRP3, 1 - in TNFRSF1A, 1 is compound heterozygote for mutations in MVK.
Conclusion: We confirmed different HAIDS in 22,5% of suspected cases. Also 10,3% of arthritis patients had HAIDS defects. Our findings demonstrate that patients with clinical diagnosis of soJA may suffer from autoinflammatory diseases missed because of similar symptoms. We recommend screening of mutations in NLRP3, MVK and TNFRSF1A for all patients with HAIDS-like clinical findings.

J07.15

The first report of dominantly inherited beta-thalassemia caused by a novel elongated β-globin chain

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A distinct set of mutations in the β-globin gene leads to the dominantly inherited β-thalassaemia that is associated with a disease phenotype even when presents in a single copy. We describe the molecular and the hematological characteristics of a novel elongated beta globin chain in combination with a known hemoglobin variant (Hb N-Baltimore) in cis. This novel β-globin variant has eight extra residues in the second exon of beta globin gene. This was determined by sequencing of amplified DNA. The produced highly unstable hemoglobin variant gives rise to typical features of major or intermedia β-thalassaemia in two members of a family depends on their genotype of alpha globin genes. The mutant beta allele of mother is transmitted in an autosomal dominant fashion to her daughter. They resemble severe forms of β-thalassaemia due to the ineffective erythropoiesis. Similar clinical features have been identified in families of dispersed ethnic origins but none in Iran. Taken together with previously published data, this result indicates that dominantly inherited β-thalassaemia should be regarded as a phenotypic term of hemoglobinopathies caused by β-chain variants that are highly unstable.

J07.16

Detection of c.657_661del5 mutation in NBN gene in patients suspected of Nijmegen breakage syndrome from Serbia and Montenegro

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The Nijmegen breakage syndrome (NBS) is a rare DNA repair disease with high risk of malignancy. The patients affected with NBS usually have microcephaly at a birth and later, growth retardation and immunodeficiency. The main cellular feature of NBS is an increased sensitivity to radiation and radiomimetic agents such as bleomycin (BLE). *NBN* gene mapped in 8q21 region is found to be disease-causing with the major founder mutation c.657_661del5, which is the mostly present in Slavic populations. The study included 10 children with clinical symptoms of NBS, who were diagnosed and treated at the Mother and Child Health Care Institute of Serbia from July 2005 to January 2012. Molecular analysis for the presence of the c.657_661del5 mutation was carried out in all patients, using modified PCR method with sequence specific primers, on PAGE gel. Homozygosity for the c.657_661del5 mutation was detected in 7 of 10 children. Cytogenetic analysis, performed on peripheral blood cultures using BLE (1μg/ml), further revealed that there were no increases in chromosomal fragility of the rest three patients without the mutation, when compared with healthy individuals. Hence, four of 7 patients carrying the mutation, showed elevated BLE-induced chromosomal breakage (0.27 -0.81 vs. 0.02-0.13 breaks/cell) with various aberrations involving chromosomes 7 and 14. The authors propose fast and reliable PCR method for the c.657_661del5 mutation detection in patients with NBS symptoms, at first. In case when this mutation is not found, cytogenetic analysis is strongly recommended, helping with differential diagnosis of other chromosomal instability disorders.

J07.17

Crohn's disease susceptibility genes and tuberculosis

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Genome-wide association studies (GWASs) are currently a standard tool in human genetics which allow identifying new candidate genes of susceptibility to complex diseases. Taking into account shared mechanisms of pathogenesis, the results of GWAS for immune-mediated diseases can be used to look for new candidate genes of susceptibility to tuberculosis (TB). Based on this hypothesis, we carried out an analysis of association between TB in ethnically different Siberian populations (Russians and Tuvinians) and genetic polymorphisms associated with Crohn's disease according to results of a GWAS (Nature, 447:661-678). Overall, 453 Russians (304 TB patients and 149 controls) and 501 Tuvinians (238 TB patients and 263 controls) were recruited. Genotyping of single nucleotide polymorphisms (SNPs) associated with Crohn's diseases in published GWASs was done using iPLEX mass-spectrometry assays. In a pilot analysis, in Russians, five polymorphisms were nominally associated with TB (p = 0.008-0.021), including rs2872507 (ORMDL3), rs3810936 (TNFSF15), rs10192702 (ATG16L1), rs9286879 (1q24.3), and rs10507523 (13q14.11). In Tuvinians, an association between TB and two SNPs, rs1407308 (TNFSF15) and rs1736135 (21q21.1), was identified (p = 0.002-0.003). The associations become non-

significant after stringent Bonferroni correction for multiple testing; however, none of the polymorphisms was previously known to be associated with TB, and their further studies in extended samples may be fruitful. Thus, the analysis of the polymorphisms associated with Crohn's disease can help identify new TB candidate genes.

J08.01
Clinical and molecular delineation of the emerging 10q22.1q22.3 microdeletion syndrome

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Five patients with interstitial 10q22 deletion have been reported so far. We report on the characterisation of an overlapping interstitial 10q22.1q22.3 microdeletion. The patient, a 2.5-years-old female, was born from the uncomplicated pregnancy at full term in a normal delivery. Her birth weight was 3156 g, length 50 cm, the Apgar scores 9/10. Hypotonia and ptosis were observed from birth. Internal hydrocephaly was diagnosed in infancy. The patient's psychomotor and language development was delayed - she could sit unsupported at age of 9 months, and still does not walk independently. During the physical examination at age of 2.5 years her height was 79 cm (<3 centile), weight 12 kg (3 centile), OFC 50 cm (75 centile). The phenotype was remarkable for ptosis, hypertelorism, epicanthus, short palpebral fissures, low set and rotated ears, short nose, and umbilical hernia. The elevated concentrations of lactate and alanine in plasma were detected. Array CGH (105K) revealed a de novo 5.18 Mb deletion of 10q22.1q22.3 (position 74236933-79422266, build GRCh37). The deletion involves 49 genes. MICU1 and MRPS16 genes are associated with mitochondrial function and their haploinsufficiency might be the cause of mitochondrial phenotype. These clinical and molecular findings increase our understanding of the clinical presentation of 10q22.1q22.3 microdeletion.

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J08.02
Whole exome sequencing in a Tunisian family with unsuspected Cohen syndrome

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Cohen syndrome is an autosomal recessive disorder associated with multiple clinical manifestations including developmental delay, acquired microcephaly, myopia, pigmentary retinopathy, joint hypermobility, truncal obesity, friendly disposition and intermittent neutropenia. In young patients, the diagnosis is difficult, because several of the characteristic features may not be present until school age or later years and the intermittent neutropenia is not always detectable. In Cohen Syndrome only the *VPS13B* (vacuolar protein sorting 13, yeast, homologue of B) gene is known to harbour causative mutations including nonsense, missense, indel and splice-site variants. *VPS13B* is required for Golgi integrity and is involved in intracellular protein trafficking. We describe the application of whole exome sequencing (WES) in a Tunisian family with two young children with developmental delay, hypotony, ptosis and thick hair and eyebrows. WES identified the presence of compound heterozygous mutation in *VPS13B* inherited from healthy heterozygous parents, confirming an unsuspected clinical diagnosis of Cohen Syndrome. In conclusion the diagnosis of Cohen Syndrome remain very difficult because of the heterogeneity of clinical manifestations in fact the introduction of exome sequencing approaches improves the diagnosis of rare diseases and helps clinicians in elucidating their etiologies.

J08.03
Leukemia inhibitory factor gene polymorphism rs929271 is associated with mild intellectual disability

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The causes of intellectual disability (ID) vary with the severity of the condition: moderate-to-severe ID (IQ<50) is much more likely to be due to a single pathological cause (genetic or environmental) whereas mild ID (IQ 50-70) is rather complex condition in origin.

Leukemia inhibitory factor (LIF) was found to regulate the neuronal phenotype and coordinate astrocyte, oligodendrocyte, microglia, and inflammatory cell responses. The T to G transversion rs929271 is located in the 3`-UTR of the LIF gene and suggested to reduce mRNA stability. Previous reports demonstrated that rs929271 may produce susceptibility to hebephrenic schizophrenia and deterioration of working memory function.

Aim of this study is to evaluate the possible association of LIF gene polymorphism rs929271 with mild ID. The group of mild ID patients consisted of 64 individuals including 40 (62.5 %) males and 24 (47.5 %) females, where previous extensive genetic investigations revealed no abnormalities. The control group consisted of 238 healthy volunteers. It was determined that total frequency of hetero- and homozygous carriers of LIF minor allele (G), is reliably higher (p=0.01) in the ID patients group (71.9%) compared to the control group (55%). The G-allele occurred less frequently 0.328 - in the control group than 0.445 - in the ID patients group (p=0.02). It was shown that the risk of mild ID development increased for both hetero- and homozygous carriers of minor G-allele and the odd ratio was 2.09 (95% CI: 1.14-3.81).

Therefore, we propose LIF as a new marker of genetic susceptibility for intellectual disability.

J08.04
Influence of mutation type and X chromosome inactivation on Rett syndrome phenotype in Czech patients

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Introduction: Rett syndrome (RTT) is a severe X-linked dominant neurodevelopmental disorder caused by mutations in MECP2 gene. The severity of clinical features is highly variable and tends to depend on specific mutations. Variation in X chromosome inactivation (XCI) is another factor, often causing milder or more severe phenotypes.

The purpose of this study was to examine whether specific mutations and XCI pattern can explain different clinical manifestations in selected Czech patients with RTT.

Subjects and methods: The severity of clinical features was evaluated in two pairs of patients with a confirmed mutation: P1 and P2 had a "mild" mutation p.Arg133Cys; P3 and P4 had a "severe" mutation p.Arg255*. XCI analysis was performed by a common human androgen receptor assay.

Results and discussion: Both patients with p.Arg133Cys had random XCI (49:51 and 43:57). P1 presented with a more severe classic form of RTT, while P2 had a mild variant typical for this mutation. P3, carrying p.Arg255*, had skewed XCI (17:83) and in comparison with P4, who had random XCI (59:41), had considerably milder phenotype regarding the age at onset of symptoms and absent epilepsy. Both patients with the p.Arg255* mutation were more severely affected than patients with the p.Arg133C mutation.

Conclusions: Our results support the hypothesis that specific MECP2 mutations and XCI influence the clinical severity of RTT phenotype, but not in all cases. Exceptions to the rule point out the need to proceed with caution in clinical prognosis and suggest that other modifying factors are involved in RTT pathology.

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J08.05
α-ketoglutarate dehydrogenase mutations may predispose Alzheimer's disease

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Introduction: α-ketoglutarate dehydrogenase (αKGDH) enzyme is a key enzyme of Krebs cycle and its mutations are known to result in extensive oxidative stress and cell damage. The hereditary mutations lead to a variety of phenotypes, extending from the lethal infant-type enzyme defect to the elderly's chronic diseases. Reduced enzyme activity has been reported in Alzheimer's patients' fibroblasts. Our objective was the mutation analysis

of the αKGDH enzyme.
Materials and Methods: Dihydrolipoamide S-succinyltransferase (DLST) and dihydrolipoyl dehydrogenase (DLD) genes (E2 and E3 components of αKGDH) were sequenced from 22 Alzheimer's patients' blood samples, from 11 Alzheimer's patients' post mortem brain tissues, and from 2 control samples.
Results: We found 4 pathogenic mutations in 3 of 11 Alzheimer patients' brains: Arg263His missense mutation in DLD gene, Pro204Leu, Leu394Met and Leu453His missense mutations in DLST gene. No mutations were present in the blood samples.
Conclusions: The presence of the 4 pathogenic mutations in the αKGDH may indicate that this enzyme is a keyplayer in the pathogenesis of Alzheimer disease. Further studies are needed to support our hypothesis.

J08.06
Breakpoint within the minimum critical region of the 15q24 microdeletion syndrome in a patient with an inherited rearrangement

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Introduction
The 15q24 microdeletion syndrome is major characterized by global developmental delay, mild-moderate intellectual disability, facial dysmorphisms, genital and digital anomalies and growth retardation. We here present a father and his daughter bearing an inversion breakpoint within the minimum critical region of this syndrome.
Patients and Methods
Both patients shared facial dysmorphic features (long face, wide forehead, depressed nasal base, pointed chin) and showed intellectual disability, more severe in the daughter. Additionally, the father manifested hiposmia and the daughter had behavioural/relation problems. Conventional and molecular karyotypes were performed. FISH experiments were completed using BAC clones for breakpoints approach at chromosome 15q.
Results
The daughter´s karyotype was 46,XX,inv(15)(q21q24)pat. aCGH revealed no genomic imbalances. FISH analysis showed that the RP11-586G22 BAC probe spanned the translocation breakpoint at 15q24.2, involving 5 genes (COMMD4, NEIL1, MAN2C1, SIN3A and PTPN9). At 15q21.1 band, the breakpoint was delimited between RP11-138E16 and 142O17 BAC probes, involving 4 genes (SECISBP2L, COPS2, GALK2 and FAM227B).
Conclusion
We speculate on the potential pathogenicity of the genes contained in 15q24.2 region that are likely to be disrupted by the inversion breakpoint and responsible for specific clinical features of the 15q24 microdeletion syndrome. Loss of NEIL1 has been reported to cause defects in olfactory function in mice, as found in the father. In neurons, SIN3A has been described to participate in learning and memory processes. The impairment of this gene might be implicated in the intellectual disability presented in our patients and those affected by the 15q24 microdeletion syndrome.

J08.07
Array CGH approach for neurodevelopmental disorders in Romania

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Background: Array-CGH approach has dramatically increased the detection rate of genomic imbalances, increasing the diagnostic yield also in neurodevelopmental disorders; however in Romania it is in most cases expensive or unavailable. This approach can be challenging with regard to interpretation of copy number variants of uncertain significance or variants with reduced penetrance.
Aim: We audited the results from array CGH in a series of Romanian children with neurodevelopmental disorders, to assess the proficiency of the method considering its costs.
Method: The Array CGH analysis of DNA were carried out in London, using oligonucleotide arrays with ~60,000 probes across the genome of 20 Romanian children with neurodevelopmental disorders. This work was done under the frame of European Social Fund, Human
Resources Development Operational Programme 2007-2013,project POSDRU/159/1.5/136893.
Results: The overall diagnostic yield of causal abnormalities was 30%. The overall yield of noncausal abnormalities was 10%. 20% came back without abnormality, while 40 % located findings that may represent a benign copy number variant, however genetic counselling was considered appropriate and blood samples from patents were requested.
Conclusion: When interpreting a result form array CGH, distinguishing the characteristics of copy number changes in relation to their clinical relevance is essential. Nonetheless, the method provided a better understanding the genetic architecture of the heterogenic neurodevelopmental disorders, thus advancing diagnosis, prognosis, and counselling for patients and families.

J08.08
Molecular cytogenetic characterization of terminal 14q32 deletion

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Detailed molecular-cytogenetic studies combined with clinical characterization are needed to establish genotype-phenotype correlations for specific deletion syndromes. Although many patients with subtelomeric deletions have been reported, the phenotype map for the terminal deletion 14q syndrome is only slowly emerging. A 14q32.3 terminal deletion with the breakpoint 14q32.31-14q32.33 only has been reported in nine cases. Here, we report on a 3 year old patient with partial monosomy of 14q32.3. She was born of healthy non-consanguineous parents, at 38 weeks'of gestation with a birth weight and length of 2300g and 44cm respectively. Head circumference at 3 years old was 44.5cm. Deletion size determined by molecular karyotyping (aCGH) is 14:102254905- 107240840 ~ 5Mb, which was confirmed by FISH. Major clinical features were presented with profound mental retardation and dysmorphisms, including broad forehead, depressed nasal bridge, pointed chin, hypotonia, epicanthus, excessive knee points extension in the standing position and walking ataxia, weakness of ligamentous apparatus. Based on our findings and review of the literature, we refine the phenotype map for typical clinical findings of the terminal deletion 14q including exclusively 14q32.31-14q32.33 bands. There is a phenotypic discrepancy in few reported so far cases, where the minimal region was 0.3Mb. Our patient hasn't congenital heart defects, genitourinary malformations and coloboma. Combining this phenotype map with benign copy-number variation data available from the Database of Genomic Variants, we propose terminal deletion 14q involving approximately 75 annotated genes. These findings should contribute to delineate the emerging phenotype in terminal 14q32 deletion but need further data.

J08.09
Microdeletions Screening of Bulgarian Patients With Developmental Delay

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Introduction: Microdeletions and microduplications are known cause for developmental delay (DD) and mental retardation (MR). MR/DD affect 1 to 5% of the general population. Establishing a genetic diagnosis in patients with MR/DD is a challenge. These small changes are not always detectable by routine karyotyping. Nowadays new molecular genetic approaches such as MLPA and array CGH allow precise detection of specific submicroscopic deletions or duplications. Next generation sequencing (NGS) could clarify diagnosis of patients with normal aCGH profile.
Materials and Methods: Over a period of 4 years 355 patients with syndromic MR of different severity were screened for deletions/duplications with MLPA. We used MLPA probe mixes P245 for microdeletion syndromes and P070/P036 for subtelomeric regions. Further analysis with aCGH was performed for 29 patients. Two patients were additionally analyzed by Illumina TruSight Inherited Diseases panel.
Results: In 56 patients (15.5%) out of 355 we were able to establish a genetic diagnosis. 44 of all aberrations (12.4%) were detected by MLPA and 4 of them were further characterized by aCGH. In 10 cases (2.8%) aberrations were detected exclusively by aCGH. Among 29 patients screened by aCGH pathogenic CNVs were detected in 14 cases (48%). In 2 additional patients diagnosis was established by NGS.
Conclusion: We present MLPA as an adequate screening procedure for clinically recognizable conditions. Array CGH presents an opportunity to investigate cases with appropriate chromosomal phenotype, where MLPA was insufficient. In our study, aCGH allowed detection of several pathogenic CNVs outside known regions. NGS provides an opportunity to diagnose cases, outside the scope of MLPA or aCGH.

J08.10

Research on development of children with genetic pathologies associated to mental deficiencies

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Introduction: Hereditary mental deficiencies (HMD) represent a group of genetic illnesses with different level of mental retardation. Most characteristic for HMD is decreasing or substantial reducing of psychical activities witch determine a row of deregulations of adaptation processes and mechanisms of organism and its existence in society. Medical-genetic and psycho-pedagogic assistance should have priority among all resources of amelioration of state of persons affected by mental deficiencies of genetic etiology.
Scope of research consists in studying of development process of children with genetic pathologies associated to mental retardations in first three years of their life, using medical, psychological and pedagogic resources of amelioration.
Materials and methods: The study included children diagnosed with genetic pathologies associated to mental retardations in first three years of life.
Results: Research results demonstrate persistence of severe retardation of children with genetic pathologies in psycho-medical aspect, related to insufficient development of all psychical processes and spheres. Psycho-physical retardation of children with genetic pathologies is not so clear during first year of life, comparing with normal children, due to biological resources and to inborn potential of individuals. Deficiency of children with genetic pathologies becomes more evident after the age of one year old. Cognitive-verbal behavior is most affected to children with genetic syndromes, followed by psycho-motional and social-affective behaviors.
Conclusion: Development of psycho-motional sphere represents an important premise for achieving sustainable increasing of formation-development of other neuro-psychic spheres. Psycho-pedagogic models of early differentiated development, including psycho-pedagogic individual program of formation, had a fundamental contribution to development of motricity, of social - affective sphere and of speech.

J08.11

15q11.2(BP1-BP2) deletion as neurocognitive disease risk factor

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Microdeletions occurred between BP1 and BP2 of 15q11.2 Prader-Willi/Angelman critical region are described in literature as the novel Burnside-Butler Syndrome.
There are four highly conserved and unimprinted genes within the 15q11.2(BP1-BP2) region:*TUBGCP5,CYFIP1,NIPA1, and NIPA2*.
TUBGCP5 is expressed in subthalamic nuclei, *NIPA1*, *NIPA2* and *CYFIP1* are expressed in central nervous system.
Haploinsufficiency for *TUBGCP5*, *NIPA1*, *NIPA2* and *CYFIP1*, without Prader-Willi syndrome, can explain some clinical features as delayed motor and speech development, dysmorphism and behavioural problems.
We describe a case of a 5 old boy with a BP1-BP2 microdeletion of 443kb spanning these four genes.
He had developmental delay, awkwardness of motor skills, behavioural problems, speech and language impairment and was referred for autism clinical suspicion.
No dysmorphism was observed in the patient.
aCGH showed a 15q11.2 microdeletion flanked by BP1 and BP2.
MLPA analysis, performed on the trios, confirmed the microdeletion in the patient and his normal mother.
The penetrance of15q11.2(BP1-BP2) deletion is estimated about at 10%. Our case provide a further evidence to support this CNV as a susceptibility locus for a spectrum of neurodevelopmental disorders.

J08.12

A NOVEL ANKRD11 MUTATION CAUSE MILD KBG SYNDROME

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Background: KBG syndrome is an autosomal dominant disease characterized by intellectual disability, seizures, short stature, skeletal anomalies and distinct craniofacial features. It is caused by mutations in ANKRD11 gene. So far, nearly 30 mutated KBG patients are reported but the range of neurologic manifestations appears extremely broad.
Methods: during regular genetic counseling we have seen an outpatient, aged 28 years, with macrodontia and distinct craniofacial dysmorphism. Direct sequencing of ANKRD11 coding regions was performed by Sanger method.
Results: a novel c.2130delG heterozygous mutation leading to a premature frameshift p.Trp710Cysfs*9 was identified in ANKRD11. Detailed clinical features included macrodontia of upper incisors, sensorineural/mixed hearing loss, hypoplastic lower ribs, brachydactyly with fifth finger clinodactyly, bicuspid aortic valve. Surprisingly, his neurologic evaluation did not reveal cognitive delay and he was of normal stature.
Conclusion: in nearly all patients with KBG intellectual disabilities are in the mild to moderate range. Single mutations in ANKRD11 have been also reported in idiopathic intellectual disability highlighting a major role for this gene in cognitive impairment and brain disorders. The present patient emphasizes the variability, especially with respect to the neurologic phenotype, related to ANKRD11 mutations allowing more accurate genetic counseling for our patients and their families.

J08.13

Challenges in medical genetics: exome sequencing uncovers recessive mutations in two cases with de novo cnv.

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De novo CNV may represent a challenge for the clinical geneticist. We report on two cases with non-syndromic intellectual disability and developmental delay (ID/DD) carrying a de novo rearrangement involving a single gene: a deletion spanning the ARHGAP12 gene, and a duplication spanning the CNOT6 gene. ARHGAP12 encodes for a member of a large family of proteins that activate Rho-type GTPase. Other genes of the same family are involved in ID or play a role in the developing axons and growth cones, suggesting ARHGAP12 was a candidate for the disease. CNOT6 encodes a subunit of the Carbon Catabolite Repressor Protein 4 (CCR4-NOT) core transcriptional regulation complex. Given the role of regulatory complexes in the pathogenesis of ID/DD, and the widespread expression of this gene, we considered its duplication as a variant of unknown significance. Because of parents consanguinity, we decided to perform a whole exome sequencing (WES). In the first family, we found a reported nonsense mutation p.Arg377* in TRAPPC9, encoding a protein implicated in NF-kB activation. Mutations in TRAPPC9 cause non-syndromic ID associated with a small head circumference, mild cerebral white matter hypoplasia, and corpus callosum hypoplasia. Our patient presents a compatible phenotype. In the second family, we identified a missense homozygous change c.154C>T (p.Cys52Arg) in VLDLR predicted as deleterious. Mutations in this gene have been reported to cause cerebellar ataxia, mental retardation and disequilibrium syndrome type 1, with a phenotype overlapping that of our patient. Our data suggest that de novo array-CGH variants should be looked suspiciously in consanguineous cases, where WES may become mandatory in future.

J08.14

Combined analysis of linkage and exome sequencing identifies a novel elongation factor associated with intellectual disability and delayed motor development

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Introduction:
Combined analysis of homozygosity mapping and whole exome sequencing (WES) has served as a powerful tool to detect pathogenic variants associated with recessive disorders. Herein, we applied a two-step approach,where linkage analysis and homozygosity mapping were used to limit exome variants to specific chromosomal regions.
Material and Methods:
We ascertained a consanguineous family from Turkey with three affected children having intellectual disability (ID) and delayed motor development. Whole genome SNP genotyping was conducted for all available family mem-

bers followed by linkage analysis. WES was performed only for one affected individual.
Results:
Two linkage peaks on chromosomes 8 and 9 were obtained with maximum LOD scores of 2.65 and 2.25, respectively. Homozygous haplotypes with identical by descent inheritance were valid only for chromosome 8. Variants were filtered from WES data solely for the linkage region on chromosome 8. Amongst, novel variants with pathogenic affect on protein were prioritized. This combined effort has led to identification of one nonsense and two missense variations in three distinct genes. All variants segregated with the condition in the family and none of them were detected in almost 480control individuals from Turkey. In silico analyses predicted benign effect for the missense variants. We therefore identified the most likely variant that caused an early stop codon in a translation elongation factor.
Conclusions:
We have identified a novel gene that encodes a translation elongation factor associated with ID delayed motor development and seizures using linkage analysis and WES.
This study was supported by TUBITAK and IU-BAP (Project Numbers: 113S331 and 11021)

J08.15
Identification of a nonsense mutation in PIDD in Pakistani families with non-syndromic autosomal recessive intellectual disability using exome sequencing

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Intellectual disability (ID) is a genetically heterogeneous neurodevelopmental disorder affecting 1-3% of the general population. It is characterized by deficits in memory skills and language development with difficulty in learning and problem solving. Today, more than 50 genes were detected in patients with ID. In an effort to identify the disease-causing mutations in patients with non-syndromic autosomal recessive ID (NS-ARID) using homozygosity mapping following by exome sequencing, we detected a homozygous nonsense mutation, c.2587C>T, p.Gln863*, in the gene PIDD in two large consanguineous Pakistani families. Sanger sequencing analysis showed complete segregation within the two families with multiple affected individuals. Analysis of cryptic relatedness was performed on a set of 73,306 independent autosomal markers in PLINK version 1.07. The pair-wise PI_HAT values between members of two families were less than 0.10 indicating that these two families were not closely related. P53-Induced Death Domain Protein (PIDD) is a Leucine-rich repeat and death domain-containing protein expressing in brain, with a key role in DNA damage/stress response. It acts as a cell-fate switch through interacting with a pro-survival molecule, RIP1 and activation of NF-kB signaling to repair and survive the lesions, or via interacting with a pro-death factor, RAIDD and activation of caspase-2 and apoptotic cell death. The p.Gln863*mutation was in the death domain (DD), through which PIDD interacts with other DD proteins such as RIP1 or RAIDD. Functional studies are ongoing to establish the mechanism of pathogenicity of the variant detected. Our finding suggests the involvement of new pathways, i.e. PIDD-related pathways, in the etiology of intellectual disability.

J08.16
A missense variant in Oligophrenin-1 segregating with intellectual disability without cerebellar hypoplasia and distinctive appearance in males from a large pedigree

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X-linked intellectual disability (XLID) is a heterogeneous condition with more than 110 genes identified. Oligophrenin-1 is a protein with a Rho-GTPase-activating domain required in theregulation of the G-protein cycle. Mutations in the oligophrenin-1 coding gene, OPHN1,usually cause XLID with cerebellar hypoplasia and distinctive facial appearance.
We studied a large pedigree including 8 males affected with moderate intellectual disability. All affected males had no distinctive dysmorphic features and the MRI showed a normal cerebellum. We first performed a haplotype-sharing analysis using SNP-array in affected males, followed by whole exome sequencing in one affected male. Four deleterious mutations were evidenced in 4 distinct genes,namely FAAH2, MAGEE1, TAF9B and OPHN1, located in the haplotypic minimal region shared by affected males. After Sanger sequencing, only one variant in OPHN1,namely R243W,was found to be co-segregated with the disease in all affected males and was absent in normal males. This variant altered a highly conserved residue located between the BAR and the PH domains of the oligophrenin protein,was predicted to be damaging by SIFT and Polyphen and was not listed in the EXAC database. Although there is currently no functional test to definitively validate the deleterious effect on the oligophrenin function of this variant, we suggest that this variant is pathogenic, expanding the spectrum of OPHN1 variants to non-syndromic XLID.

J09.01
Novel and recurrent mutations in NF1 gene causing neurofibromatosis in Bulgarian patients

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Neurofibromatosis type 1 (NF1) is characterized by skin pigmentation (cafe-au-lait spots), Lisch nodules and growth of tumors along nerves in the skin, brain, and other body parts. NF1 is an autosomal dominant disorder, caused by mutations in the *NF1* gene (17q11.2). The produced nonfunctional neurofibromin cannot properly regulate cell growth and division. Segmental NF (NF5) is a rare form in which multiple neurofibromas are restricted to a single body part without the typical characteristics of neurofibromatosis.
Here we report 15 Bulgarian NF1 cases and one segmental NF5 case. All but one of NF1 cases presented „café au lait" macules. Plexiform neurofibroma(s), Lisch nodules, sphenoid wing dysplasia and freckling in the axillary and inguinal regions were reported in some cases. The segmental neurofibromatosis case suffered by multiple neurofibromas formation in the right thenar hand, engaging thumb and middle finger. The age of onset is early after birth.
Mutations in the *NF1* gene were detected in nine patients (56%). Three mutations are novel, two germline and one somatic. The germline mutations were detected in blood: a splice site mutation c.4725-1G>A and deletion of exons [41-52]⋯56. The case with segmental neurofibromatosis was tested negative for *NF1* mutations in blood but a somatic deletion of exons [1-12] was detected in fresh neurofibrom biopsy.
In conclusion, the detection of mutations in *NF1* gene allows family planning, better genetic counseling and prenatal diagnostics in affected families. The cases with segmental neurofibromatosis must be genetically interpreted with caution, as they are caused by somatic mutations in neurofibroms.

J09.02
Novel mutation in SPG4 gene in patients with spastic paraplegia from Bashkortostan Republic (Russia)

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Hereditary spastic paraplegias (HSP) - is a genetically and clinically heterogeneous group of neurodegenerative disorders characterized by progressive spasticity of the lower limbs. The major pathological feature of HSP is degeneration of pyramidal tracts. Autosomal dominant (AD), recessive (AR), or X-linked inheritance were described. To date, 30 causative genes and 50 loci have been identified. Mutations in the SPG4 gene are responsible for about 45% of the pure AD type of HSP and for 12-18% of the sporadic cases. SPG4 codes for spastin - AAA (ATPase associated with diverse cellular activities) family protein. Over 300 mutations have been described.
The HSP frequency in Bashkortostan Republic (BR) is 3,5:100000. We examined HSP patients from BR and detected one mutation in patients from one large Bashkir family in SPG4 gene. This previously undescribed frameshift mutation is deletion of ten nucleotides in exon 6 leading to the formation of a premature stop codon in 15 codons after mutation codon (c.885del10 (p.Thr295ThrfsX16)). Disorder in this family has autosomal dominant mode of inheritance. The disease in proband and his relatives manifestated at the beginning of the third decade of life. The first symptoms were lower extremity spasticity and gait disturbance. Clinical examination revealed lower central spastic paraparesis with increasing hypertonicity at the initiation of movement in proband at the age of 47 years. An absence of any other neurological symptoms allowed concluding that there is pure form of HSP in this family. The mutation was not revealed in the control group of Bashkir ethnicity.

J09.03

A severe neurological syndrome of ventriculomegaly, agenesis of corpus callosum, psychomotor retardation and seizures mapped to a locus on chromosome 12.

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Large consanguineous Bedouin family of the Negev area of southern Israel presented with an autosomal-recessive severe neurological syndrome of ventriculomegaly, agenesis of corpus callosum, psychomotor retardation and seizures. Patients karyotype, chromosomal microarray and metabolic workup were normal. Homozygosity mapping using Affymetrix 250K SNP arrays identified a single ~7Mb disease-associated locus on chromosome 12 between rs10734905 and rs903770. Multipoint LOD score was calculated and yielded a score of 3.82 at rs7312321. Within the pre-defined locus are some genes associated with neuronal development. Mutations in these genes are known to cause severe phenotypes similar to those presented by our patients: HSPB8 (MIM#158590), COX6A1 (MIM#616039), ACADS (MIM#201470), C12orf65 (MIM#615035), EIF2B1 (MIM#603896) and TCTN2 (MIM#603896). Whole exome sequencing was performed and data analysis is being processed.

J09.04

Familial pericentric inversion 18: A new recombinant child

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Introduction
The pericentric inversions frequency of the population is 1-2%. In most of the inversion carrier, the phenotype is normal, but carriers of inversions are at risk of having unbalanced offspring due to meiotic crossing-over. The risk of having an abnormal child is about 5-10 %. Here, an unbalanced offspring whose mother is pericentric inversion 18 carrier was presented.
Material and Methods
Cytogenetic analysis and SNP Array were performed on the patient's peripheral blood.
Findings
1 year old female patient had minor dysmorphic feature and epilepsy. Her mother had oligohydramnios in prenatal period. The neuro-motor development was normal. She was diagnosed with epilepsy when she was 8 month old. The patient's mother was known to be familial inversion 18 carrier.
Cytogenetic analysis was performed on the patient's peripheral blood by GTG banding method. Her karyotype was 46,XX,inv(18)(p11,2;q21) like her mother. SNP Array was performed on the patient's peripheral blood and a heterozygote deletion which was 1,177,294 base pairs in size on 18p11.31 chromosomal region was found. This region contains C18orf42, LINC00526, LINC00667, ZTBD14, EPB41L3, LOC645355, MIR3976, TMEM200C, L3MBTL4, MIR4317, C18orf64, ARHGAP28, LINC006, LAMA1 genes. It is suggested that this heterozygote deletion is responsible for the patient's clinical findings. There were children with severe abnormalities and died early due to inv18 in this family. Genetic counseling was given to family. PGD was recommended in subsequent pregnancies.
Conclusion
18p11.31 deletion is associated with normal phenotype to mild dysmorphic feature, autism, epilepsy and intellectual disability in the literature. Our findings were similar to the literature.

J09.05

The clinical peculiarities of congenital malformation of the brain

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Introduction: Cerebral malformations represent a great problem in neurogenetics. More than 2000 different congenital malformations of the brain have been described in the literature, and their incidence is reported to be about 1 percent of all live births. In congenital brain malformations development involved several factors: genetic factors, vitamin deficiency, infections, irradiation factors, medications, etc.
Materials and methods: The study included 55 patients (25 girls and 30 boys) aged between 2 week - 12 month, diagnosed with congenital brain malformation (CBM).
Results: All the children included in the study were examined by complex neurological examination and supplementary investigation: neurosonography, computed tomography (CT) - brain and magnetic resonance imaging (MRI). It was concluded that agenesis of the corpus callosum was the most common congenital brain malformation in children aged under 1 year (42%), microcephaly (27%), agenesis of the septum pellucidum (11%). The most rare congenital brain malformations were: hydranencephaly (5%), anencephaly (5%), hemimegalencephaly (2%) and microgiria (2%). The most common clinical manifestations were: neuropsychic retardation of varying degrees (91%), disorders of muscle tone (50%), partial seizures / infantile spasms (36%).
Conclusion: A congenital malformation affects neurological development and reduces the ability of the children. The prognosis of the children with CBM varies depending on the type and severity of the defect: some congenital defects causing minor neurological disorders, others are so severe that they are fatal before or soon after birth.

J09.06

Analysis of solute carrier family 6 member 4 gene promoter polymorphism in young Turkish basketball players.

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Serotonin transporter (5-hydroxytryptamine transporter, 5- HTT) gene (SLC6A4) is considered to be one of the most important candidate genes for genetic involvement in psychiatric conditions like anxiety, self- confidence and motivation. In the present report, we aimed to analyze the distribution of SLC6A4 promoter long and short (L and S, respectively) polymorphism in young Turkish basketball players. We enrolled 24 players in the study, 17 were females and 7 were males. 12%, 35% and 53% of the females had SS, LS and LL genotype, respectively; whereas 28,5% of the males had SS and the same percentage of them had LS, and 43% had LL genotype. When we examined the allelic counts, L allele was recorded as 71% in females and 57% in males; S allele was 29% in females and 43% in males. Our results were in agreement with the previous ones, indicating the presence of L allele in individuals dealing with sport. We suggest that SLC6A4 promoter analysis is important for genetic counseling for the individuals who are prone to be successful in sports.

J09.07

Methylation status and calorimetric profiles of serum proteins in schizophrenia patients

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Background: Schizophrenia is severe chronic psychiatric disorder. It has multifactorial etiology. Epigenetic factors such as DNA methylation could be involved in the schizophrenia pathogenesis. Environmental factors could modify the gene expression and change the thermodynamic behavior of blood sera.
Materials and Methods: We have performed high-resolution genome-wide methylation array analysis on pool samples consisted of 220 patients and 220 healthy controls to determine the methylation status of 27,627 CpG islands. The effect of environmental factors on gene expression was analyzed by differential scanning calorimetry (DSC) applied on 92 patients and 21 controls.
Results: We found 394 differentially methylated regions (DMRs) between schizophrenia patients and control DNAs. In the promoters were discovered 146 CpG islands (96 hyper- and 50 hypomethylated). The potential new discovered candidate genes with DMRs participate in transcription regulation, neurotransmission, cell motility and adhesion and nervous system development. Significant thermodynamic modifications in blood sera behavior of schizophrenia patients were revealed. The calorimetric profiles were classified in four distinct groups, reflecting different thermal stabilization of the high-abundance portion of the serum proteome.
Conclusion: Our data revealed substantial differences in methylation and calorimetric profiles between schizophrenia patients and controls. It is proposed that a combination of DNA methylation microarray analysis and calorimetric analysis of proteins is a perspective strategy for a comprehensive study of schizophrenia.

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J09.08

Analysis of several loci from genome-wide association studies in Parkinson's disease in three ethnic groups from Bashkortostan Republic of Russia

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We performed replication of genome-wide association analysis (GWAs) with Parkinson's disease (PD) in three ethnic groups from Bashkortostan Republic on 550 PD patients (Russians - 215, Tatars - 243, Bashkirs - 90) and 622 controls (Russians - 190, Tatars - 338, Bashkirs - 94). The study included analysis of 15 polymorphic loci: rs356219, rs356165, rs2737020, rs2619364 and rs6532194 in SNCA gene; rs11012, rs2942168, rs393152, rs1724425, rs12373139 and rs1981997 in chromosomal region of MAPT gene (17q21.31); rs1491942, rs1907632, rs34778348 (G2385R) associated with LRRK2 gene, and rs7077361 - in ITGA8 gene.
The results of the analysis in three ethnic groups were different. Genetic markers of the increased risk of PD development in Russians were alleles: rs356219*G (p = 0,006; OR = 1,58), rs356165*G (p = 0,003; OR = 1,64) of SNCA gene, rs11012*A (p = 0.007; OR = 2,12) of MAPT- region; in Tatars - rs6532194*T (p = 0,04; OR = 1,40) of SNCA gene; rs2942168*C (p = 0,02; OR = 1,60), rs393152*A (p = 0,001; OR = 1,92), rs1724425*C (p = 0,04; OR = 1,3), rs1981997*C (p = 0,01; OR = 1,72) of MAPT- region; rs7077361*T (p = 0,005; OR = 1,74) of ITGA8 gene. Polymorphsm rs34778348*G/A (G2385R) of LRRK2 gene was found only in PD patients of Tatar ethnic origin (0.6%). In Bashkirs association with PD development was confirmed only for rs1491942l in LRRK2 gene: genotype *G/G was protective against PD development of (p = 0,01; OR = 0,07).

J09.09

Genetic association of 5-HT1A and 5-HT1B gene polymorphisms with OCD in the Turkish population

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Objectives: Obsessive-compulsive disorder (OCD) is a common neuropsychiatric disorder with complex genetic etiology. We investigated the possible roles of C-1019G promoter region polymorphism of the 5-HTR1A and G861C coding region polymorphism of the 5-HTR1B receptor genes in the susceptibility to OCD in the Turkish population.
Methods: Two single nucleotide polymorphisms that are the 5-HTR1A (rs6296) and the 5-HTR1B (rs6295) receptor genes were genotyped in 76 OCD patients and 57 healthy control groups that are unrelated age-matched and sex-matched by using the PCR-RFLP.
Results: We did not observe any difference in the genotype distributions of the rs6296 and rs6295 between the OCD patients and the control groups.
Conclusions: To the best of our knowledge, our study is the first to confirm the association of the variant rs6296 that is related to HTR1A with OCD in the Turkish population. As a result of our study, polymorphisms of rs6296 and rs6295 receptor genes are possibly a weak candidate that will contribute in determination of the relationship between 5-HT1A, 5-HTR1B receptor genes and OCD.

J09.10

Association of ADARB1 gene with suicide attempt risk in patients with major psychiatric disorders

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Introduction: Adenosine-to-inosine RNA editing, catalyzed by ADAR and ADARB1, is widely used to fine-tune the functional properties of proteins key to neurotransmission, and is thought to be essential for behavior and cognition. Changes in the editing of some target transcripts and changes in ADARB1 mRNA expression were demonstrated in the brains of psychiatric suicide victims. Transgenic mice misexpressing the rat's ADARB1 isoform is a model of endogenous depression, indicating that genetically determined differences in ADARB1 expression may be involved in psychiatric pathologies. To test this, we performed an association study of common variants in ADARB1 gene with suicide attempt (SA) in psychiatric patients.
Materials and Methods: 165 suicide attempters and 188 suicide non-attempters, suffering from major depressive disorder, bipolar disorder or schizophrenia were genotyped for 11 selected tag-variants mapping in ADARB1 region coding deaminase domain and 3'-untranslated region. Associations were tested using Pearson's $\chi 2$-test and logistic regression adjusted by psychiatric diagnoses. Multiple testing bias was corrected by applying a 10e6 permutation test.
Results: Rs9983925 minor allele T decreased the risk for SA (P=0.028, OR=0.705), while the rs4819035 minor allele G increased the risk (P=0.028, OR=1.495). The genotypic tests revealed significant result only for the additive model (rs9983925: P=0.032, OR=0.710; rs4819035: P=0.042, OR=1.437). The observed associations were confirmed with permutation test.
Conclusion: This is the first study showing the association of ADARB1 with SA in psychiatric patients, implying genetically determined involvement of RNA editing in SA. The identified risk variants overlap putative 3'-regulatory regions of ADARB1 and may influence its expression.

J09.11

Association Study of a Brain-Derived Neurotrophic Factor genetic polymorphism Val66Met and Major Depression Disorder in Colombian population

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Major Depression Disorder(MDD) affects morbility, mortality and quality of life of many people around the world(1). This disease has heritability between 30% and 40%, indicating an important genetic component(2). Otherwise, it has been suggested that the Val66Met polymorphism of the brain-derived neurotrophic factor gene (BDNF) could be associated to this disorder(3). During the present investigation a case-control approach was carried out in order to determine the genotype of clinical Colombian population and their match controls. For this, a cognitive diagram of each individual was evaluated using the standard psychological tests M.I.N.I, STAI, IDER, and YSQ SF, then genotyping was performed by qPCR and data was analyzed using R software. Association studies show no effect between these variants and MDD (OR=1 Y P-value= 0,99). When psychological data was analyzed, a strong association between abuse and depression phenotype was found (OR=0.11, p=0.00036). When analyzing for interactions, between the BDNF variants and childhood abuse we observed that individuals with the Val allele and childhood abuse where more prone to develop MDD (LOD=2.83). These results suggest that Val66Met variant per se has no influence over the MDD but people with the genotype Val/Val could be more vulnerable to develop a depressive disorder when they have been abused in their childhood.
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(2) Sullivan, et al. 2000. Reviews and Overviews Genetic Epidemiology of Major Depression : Review and Meta-Analysis, (October): 1552-1562.
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J09.12

Symmetrical progressive calcification of brain basal ganglia:symptomatic or idiopathic

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Different perinatal pathologies, as well as pseudohypoparathyroidism (PHP) and genetic disorder known as Fahr's disease can cause calcification of brain hemispheres in the early childhood. In our studies of rare disorders to a special interest came a condition such as PHP, when high concentration of parathyroid hormone in the blood is associated with resistance of the organism towards biological activity of the hormone, namely, patients have hypocalcemia and hyperphosphatemia. Modern molecular-genetic methods play a significant role in child neurology clinic in relation to both diagnostics and treatment. Our presented case likely to be PHP type 1b (male, 16yo), who has developed his first partial epileptic seizures at age 13. Brain CT performed at age 15 showed symmetrical calcification in the region of basal ganglia. Mild neurological signs were observed (mild muscle hypoto-

nia, slowness in movements, a little euphoric, low school performance and restricted interactions with peers). Oral intake of carbamazepine was not effective, valproate sodium as second drug has improved seizures' control. The second CT performed at age 17 showed progression of calcification including frontal lobe. Biochemistry and molecular genetic exams confirmed a rare pathology - PHP type 1b which is characterized by molecular defect in the GNAS1 gene which is responsible for the renal resistance to PTH. The seizures identified as symptomatic and require additional treatment with active form of vitamin D3 and IV calcium to normalize blood calcium level and prevent progression of brain tissue calcification and finally decrease epileptic activity and enhance seizure control.

J09.13

A highly polymorphic copy number variant in the NSF gene is associated with cocaine dependence

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Cocaine dependence is a complex psychiatric disorder with both genetic and environmental factors involved. The involvement of different neurotransmitter systems in cocaine effects makes the molecular machinery that controls the release of neurotransmitters to the synaptic cleft a good candidate for participating in the development of the dependence. In a previous work we identified a risk haplotype for cocaine dependence in the gene encoding the N-ethylmaleimide Sensitive Factor (NSF), which is essential for synaptic vesicle turnover. Recently, another study identified a large polymorphic copy-number variant (CNV) that encompasses the first 13 exons of the *NSF* gene. We examined the possible contribution to cocaine dependence of this CNV in the *NSF* gene. For this purpose, we performed a case-control association study in a discovery sample of 359 cocaine-dependent patients and 356 sex-matched controls. Using qRT-PCR technology we genotyped individuals and analyzed the results considering two genotype groups: low number of copies (2 and 3) and high number of copies (4, 5 and 6), and identified an association between cocaine dependence and the CNV (P=0.013). We then performed a replication study consisting of a new sample of 508 cocaine-dependent patients and 569 controls, and the association persisted (P= 7.1 e-03). Finally, we did a pooled analysis and the association also remained significant (P= 1.8 e-04) with a higher frequency of individuals with low number of copies in cases (70.6%) than in controls (62.2%). These results, together with the ones obtained in a previous study from our group, support a role for the *NSF* gene in the susceptibility to cocaine dependence.

J09.14

Exploring SNPs in miRNA binding sites of genes expressed in brain as risk factors for Substance Dependence

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Drug addiction is considered a disorder of neuroplasticity in brain reward and cognition systems that results from aberrant activation of gene expression programs in response to prolonged drug consumption. Non-coding RNAs (ncRNAs), such as microRNAs (miRNAs), are key regulators of almost all aspects of cellular physiology. Recently, miRNAs were shown to play key roles in the drug-induced remodelling of the brain reward system that likely drives the emergence of addiction. In this work we aim at exploring the role of miRNAs in drug addiction. Using a case-control association approach we evaluated, in a sample of 735 drug-dependent patients and 739 sex-matched controls, the possible contribution to drug dependence predisposition of 62 SNPs in the 3'UTR of several genes expressed in the central nervous system that are predicted to alter the binding of miRNA molecules. Seven polymorphic variants were found nominally associated with drug addiction. We subsequently assessed these seven SNPs in a replication sample of 663 drug-dependent patients and 667 sex-matched controls and found association between rs1047383 in the PLCB1 gene and drug dependence. This association remained significant when we analyzed the results in the whole sample (1,392 cases and 1,403 controls), with a higher frequency of carriers of the C allele in cases (40%) than in controls (35.5%). Our results suggest that the PLCB1 gene may participate in the susceptibility to drug dependence.

J09.15

Lack of association between mitochondrial DNA T4216C variation and multiple sclerosis in an Iranian population

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Multiple Sclerosis (MS) affects nerves in the brain and spinal cord and has been the topic of global research; yet there is no commonly accepted cause and no cure for the disease. Mounting evidence supports the role of genetics in susceptibility to MS. From this perspective, a current effort focuses on the neurogenetics of the complex pathogenesis of MS in relation to factors such as mitochondrial DNA (mtDNA) variations or mutations. The present study tested whether mtDNA T4216C variation in the ND1 mtDNA gene is associated with MS in an Iranian population.
Blood samples were collected from 100 patients with MS and 100 unrelated healthy controls, and DNA extraction was carried out by means of a salting-out method. By means of appropriate primers, polymerase chain reaction (PCR) amplification was carried out for the mtDNA fragment. Afterwards, the PCR products were digested using Nla III restriction endonuclease enzyme for analysis of Restriction Fragment Length polymorphism (RFLP) in mtDNA T4216C variation. With electrophoresis by means of 3% agarose gel and *safe DNA gel stain, we imaged restriction products in a UV transilluminator.* The accuracy of genotyping procedure was confirmed by sequencing the mtDNA fragment.
No significant statistical difference in the frequency of the T4216C mtDNA variation was found between the patients (24 %) and the control subjects (21 %) (P=0.61). Logistic regression analysis showed an OR of 1.1 (95% CI=0.5-2.4).
The present study revealed no association between the T4216C variation in mitochondrial ND1 gene, and MS.
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J09.16

A family history of dementia influences the development and the progression of late-onset Alzheimer's disease

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Late onset Alzheimer's disease (AD) has no recognizable Mendelian pattern of inheritance but its development may be linked to a family history of dementia (FH). We investigated the possible role of FH in AD susceptibility, age at AD onset (AAO) and cognitive decline in a sample of Italian subjects (504 AD patients and 265 controls). FH was ascertained by interview. FH was found in 28% of AD patients and 15% of controls (p=0.0001). The degree of relationships among relatives with dementia was collected for 97 patients . Logistic regression analysis showed that FH carries an increased risk of AD (OR= 1.9,C.I. 1.3-2.8) independent from the presence of APOE e4 allele, and that FH in first-degree relatives (parents and siblings), is an even higher risk (OR= 5.97,C.I. 2.8-12.7). Among the AD patients with one parent affected, the ratio of mother-to-father with dementia was 1.7/1, confirming that maternal FH may impact on AD development. Among the AD patients with a first-degree FH, mean AAO (±sd) was lower among those with affected parents (73.6±6.8 years) than in those with affected siblings (77.2±5.7,

p=0.02). Cognitive decline (MMSE test scores) was lower in patients with affected parents (17.4 ±5.1) than in those with affected siblings (20.3 ±3.6, p=0.02). AAO and cognitive decline were lower in patients with an affected mother (73.0±7.8 and 16.6±5.3 respectively) than those with an affected father (75.0±5.2 and 18.3±4.9, respectively). In conclusion FH influences AD development and quantitative traits reflecting disease severity, including AAO and cognitive impairment.

J09.17
Case report of Prader -Willi syndrome

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Prader-Willi syndrome (PWS) is a complex genetic disorder, characterized by neonatal hypotonia, delayed development, short stature, childhood obesity, hypogonadism, characteristic facial features, and other features . It can be considered to be an autosomal dominant disorder and is caused by deletion or disruption of a gene or several genes on the proximal long arm of the paternal chromosome 15 or maternal uniparental disomy 15, because the genes on the maternal chromosome 15 are virtually inactive through imprinting. This region contains genes that are epigenetically imprinted. We report 12-years old girl with Prader-Willi syndrome. At the day of her birth, she had severe hypotonia, her physiological reflexes were very indolent, also she could not suck or swallow herself. At the age of 12 the patient was admitted for genetic counselling. All laboratory tests for newborns were performed which showed normal results, including karyotipe test (46XX). Neurosonography showed asymetrical, rounded ventricles which suggested hypoxic ischemic changes in the brain. Phenotypically, almond-shaped eyes, prominent nasal bridge and brachydactyly were observed. Moreover, defects in the internal organs, retardation and cysts in the brain were also observed. After the consultation, fluorescent in situ hybridization (FISH) cytogenetic test was carried out to verify the hypothesis of Prader-Willi syndrome. The results showed 15q11 region deletion in 100% of interphase nuclei of lymphocyte culture. The phylogenetic tree was constructed and it showed that patient's father is a carrier. This case shows the importance of genetic testing when patients are suffering from severe hypotonia.

J09.18
Analysis associations of cognitive performance genes revealed by GWAS with intellectual features in Russian population of West Siberian region

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The research of genetic factors influencing to cognitive function of healthy adults arouse of great interest. All genes that determine the intellectual abilities still have not identified.Genetic researches are based on GWAS revealed genetic variants associated with cognitive performance. The aim of this study was analyze the associations of 5 SNPs (rs8020441, rs2247572, rs2616984, rs2252521, rs2229741) are reported in GWAS with personal intellectual properties that are determined with help (16PF) Cattel's test.This traits included the following factors: B - Reasoning, M - Abstractedness, N - Privateness and Q1 - Openness to Change. Cognitive performance according to recent GWAS studies were genotyped by real-time PCR in Russian healthy adults (N = 150; 111 - women and 11 men; mean age was 22,8+/-0,10). DNA samples were genotyped using TaqMan® SNP Genotyping Assays (Applied Biosystems) under condition recommended by the manufacturer. The relationship of genotypes with quantitative factors B, M, N and Q1 of 16PF was estimated by Kruskal-Wallis ANOVA test. The associations of rs2247572 in KCNB2 gene and rs2252521 in CSMD1 gene with factor B were revealed (p<0,05). The association both SNPs with cognitive performance was reported in GWAS by Cirulli et al., 2010. Also there were established moderate associations (p<0,01) of ZNF804A gene with abstractedness (factor M) and NRIP1 gene with openness to change (Q1). Our findings are support the association of genetic variability KCNB2 and CSMD1 genes with cognitive performance in Russians.The identified associations presuppose substantial contribution of genetic variability in individual mental constitution perhaps.

J09.19
The case of combination of Glutaric acidemia type II and hyperhomocysteinemia

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Background. Glutaric acidemia type II is characterized by multiple acyl-CoA dehydrogenase deficiencies resulting in significant excretion of glutaric acid, as well as lactic, ethylmalonic, butyric, isobutyric, 2-methyl-butyric and isovaleric acids.
Case report. A., 4 year old girl, with seizures, severe delay in the psycho-speech development, is not able to sit, stand and walk, holds her head badly, chokes with liquids.
The child of 3rd pregnancy, that progressed under condition of maternal preeclampsia, threat of miscarriage. The child was born with respiratory failure, hyperthermia, and convulsions.
The survey found high levels of ammonia, hydrothorax, gidroperitoneum, MRI Dandy Walker cyst. Anticonvulsant, detoxification and antibiotic therapy did not ensure any improvement.
Phenotype: microcephaly, microangiopathy, muscle hypertonia, hepatomegaly, nephroptosis.
In the pedigree: cardiovascular and oncological diseases.
In urine increased concentration of 2-ketoglutaric acid, 3-hydroxybutyrate, 3-hydroxypropionic acid and 4-hydroxyphenylacetate, glutaric acid, ethylmalonic acid and lactate.
Blood test:
C12-0,35 (result 0.469 mM/m) -acylcarnitine
C12: 1-0,24 (0.469 mM/m) -dodekspaylcarnitine
C4-0,1 (1.58 mM/m) -izobutirylcarnitine
C5-0,6 (1,16 mM/m) -valeril-2-methyl-butylcarnitine
C6-0,24 (0.691 mM/m) -geksanaylcarnitine
C8-0,3 (0.461 mM/m) -oktancarnitine.
Homocysteine - ↑ 17,5 mmol/l
Diagnosis: Organic aciduria, glutaric acidemia type II, hyperhomocysteinemia
Conclusions: A diet with a sharp restriction of fats and proteins (1 g/1 kg of body weight), energotropic therapy and correction of metabolic disorders have significantly improved the condition of the child. Ammonia and homocysteine levels in blood have normalized; the child began to swallow normally.

J09.20
Down regulation of antioxidant enzymes in SCA28

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Introduction:
Autosomal dominant spinocerebellar ataxias (SCAs) are genetically heterogeneous neurological disorders characterized by cerebellar dysfunction mostly due to Purkinje cell degeneration. Spinocerebellar ataxia 28 (SCA28) is caused by heterozygous mutation in the *AFG3L2*
gene, on chromosome 18p11.22-q11.2. This gene encodes a component of the conserved m-AAA metalloprotease complex involved in the maintenance of the mitochondrial proteome. Altered oxidative stress response has been supposed for SCA28 as well as for other neurodegenerative conditions. In eukaryotes, antioxidant enzymes involved in reactive oxygen species (ROS) response include superoxide dismutase (SOD) enzymes, which catalyze the conversion of superoxide anions to hydrogen peroxide, and catalase (CAT), a H_2O_2-degrading enzyme. The Cu/ZnSOD or SOD1, is a cytosolic enzyme, the predominant SOD in most cells and tissues, accounting for 70-80 % of the total cellular SOD activity; the MnSOD or SOD2, is a key mitochondrial antioxidant enzyme. Materials and methods: We evaluated in SCA28 patients vs. controls lymphoblastic cell lines (LCLs) mRNA levels of CAT, and SOD2.
Results: We found that CAT and SOD2 expression were down-regulated in SCA28 patients of 20% and 15% respectively (p=0.0118; p= 0.0004). The analysis of enzymatic activity showed a 10% of reduction of SOD1
activity in SCA28 LCLs vs. controls. Conclusions: Major emphasis has been given on the role of oxidative stress and free radical chemistry with respect to major neurodegenerative disorders (e.g., AD, PD, and
ALS). Here we further suggest the role for oxidative stress in the pathogenic mechanism of more rare neurodegeneration disease such as SCA28.
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the Italian Ministry of Health.

J09.21
Analysis of mutation distribution for focused groups of children with epileptic encephalopathy

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Background: Epilepsies are highly heterogeneous with a genetic contribution. The variety of nonspecific and overlapping syndromic and nonsyn-

dromic phenotypes often hampers a clear clinical diagnosis and genetic testing, however thorough focusing patient groups can help overcome this difficulty. The aims of study: high-throughput sequencing of genes associated with epileptic encephalopathy and mental retardation (thirty-four genes in panel; focused children groups; 100 persons, including controls/parents); searching for de novo mutations and analysis of mutation distribution among studied genes. Particular attention is given to patients of first years of life with epileptic encephalopathy, resistant to anticonvulsants. Methods: DNA isolation: MagNA Pure LC2, targeted genomic enrichment: NimbleGen SeqCap system, sequencing: 454 GSJunior. All steps of the sample preparation/sequencing were performed according to the manufacturer's protocols. Results: The structure of the gene variants (are listed only missense-mutations with MAF less 0,5% or unknown) (gene/persons/mutations): SCN1A/15/10 (3 of them are nonsense-mutations; 9 are unannotated (NCBI)), SCN1B/3/1, SCN2A/2/2, SCN9A/1/1, NRXN1/8/4, ZEB2/2/1, TREX/1/1, CNTNAP2/3/2, DLGAP/6/5, SPTAN1/2/2, GRIN2A/4/4, GRIN2B/1/1, RNASEH2A/1/1, RNASEH2B/3/2, CDKL5/1/1, PCDH19/1/1, UBE3A/2/1. An about half of revealed missense-mutations are unannotated (NCBI). Long 454 reads revealed some extended structural features undetectable from NGS with shorter reads: deletions (75b.p. (SCN1A); 59b.p. (SLC9A6)), inversions (311b.p. (NRXN1)). Conclusions: The data obtained allowed to confirm clinical diagnosis for most patients studied. The research was supported by the Department of Health of Moscow.

J09.22
The role of gene x environment interactions in anxiety-related traits in healthy individuals

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Anxiety appears to be the endophenotype of affective disorders caused by gene-environment interactions. Despite multiple studies focused on associations of serotonergic system genes with anxiety-related traits, there is some controversy since multiple genes of small effect are involved. Recently, the involvement of genes encoding contactin associated protein like 2 (CNTNAP2) and neurexin 1 (NRXN1) in anxiety-related phenotypes was published (Stein et al., 2011; Grayton et al., 2013). The aim of this study is to examine the role of NRXN1 (rs4971648, rs1045881) and CNTNAP2 (rs2710102) polymorphisms contributing into anxiety variations under gene-environment interactions.

The study involved 520 healthy individuals of Russian, Tatar, Bashkir, Udmurt populations from Russia (75% women) (mean age 20,3 ± 3,87 years) without any history of psychopathologies subjected to state (SA) and trait anxiety (TA) assessment using State-Trait Anxiety Inventory . SNPs genotyping was performed via PCR-RFLP. Statistical analysis was conducted with Plink v.1.07.

Linear regression analysis revealed GxE models determining variations in anxiety. The association of CNTNAP2 rs2710102 G-allele and lower TA was observed in individuals reared by mothers with the older age (P=0.035), while CNTNAP2 rs2710102 G/G-genotype was associated with lower TA in individuals who have several sibs (P=0.0049). Moreover, higher SA was found in individuals with NRXN1 rs4971648 A-allele who were younger children in family (P=0.036) and with NRXN1 rs1045881 A-allele with high income level (P=0.031).

These findings suggest that environmental factors modulate the association of NRXN1 and CNTNAP2 genes with variations in anxiety.

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J09.23
Association analysis of Vitamin D Receptor Gene rs4334089 polymorphism with Parkinson's disease in Iranian Subjects

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Introduction: Parkinson disease (PD) is a neurodegenerative disorder with both contributions of genetic and environmental factors. Protective functions of vitamin D and then vitamin D receptor (VDR) in PD have been declared recently. With this regards in this study we investigated the association of VDR rs4334089 allele gene polymorphism in Iranian PD patients. Method and materials: DNA was extracted from 520 PD patients and 520 unrelated Iranian subjects. Evaluation of rs4334089 allele polymorphisms were done by PCR-RFLP method using NalIII restriction enzyme. PD was characterized by UK Parkinson's disease Society Brain Bank Clinical Diagnostic Criteria and study protocol was approved by our institution ethics committee. Results: Analysis of data showed that rs4334089 AA genotype (OR=1.487, 95% CI: 1.130-1.956, P = 0.003) and rs4334089 A allele (OR=1.251, 95% CI: 1.053-1.486, P = 0.011) were significantly more frequent in PD patients in compare to control group. Conclusion: Our findings suggest that VDR rs4334089 AA genotype and A allele could be important risk factors for PD in Iranian subjects. So, considering of this genetic background point could be helpful to achieve an appropriate schema for PD management.

J09.24
De novo case of a mosaic ring supernumerary marker chromosome leading to trisomy of 8p11.22-q11.23 in a boy with development delay and corpus callosum hypoplasia

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Small supernumerary marker chromosomes (sSMC) are structurally abnormal parts of the karyotype. ~70% of people with sSMC grow and develop normally, while 30% show different clinical signs and symptoms. We present the clinical and cytogenetic findings in a 6-year-old male referred for genetic evaluation because of developmental delay, behavior problems, *corpus callosum* hypoplasia, minor dysmorphic features. Cytogenetic analysis of GTG banded metaphases revealed a *de novo* supernumerary ring chromosome in 50 % of the analyzed cells. aCGH indicated a 13.8 Mb gain of 8p11.22-q11.23 including 85 RefSeq genes.

Based on gene expression and function analysis in databases, we suggest that developmental delay and *corpus callosum* hypoplasia may be caused by overdose of the *NKX6-3*, *THAP1*, *POMK*, *UBE2V2* genes. *NKX6-3* encoded protein is transcriptional repressor involved in early stages of neural crest development. The neural localization of THAP1 and its nuclear compartmentalization suggests that it may control neuronal gene transcription. The highest expression of *POMK* gene in human fetal and adult brain suggests an important role in brain development. *POMK* encoded protein-O-mannose kinase is involved in O-glycosylation and neuronal migration processes. *Ube2v2* expressed ubiquitin-conjugating enzyme is distributed widely in the rat brain in the late embryonic developmental stage and appears to have an important role in brain development and neural differentiation.

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J09.25
Expanding HAX1 mutational spectrum in Kostmann syndrome "neurologic variant"

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Kostmann syndrome (KS) or autosomal recessive severe congenital neutropenia 3 (OMIM #610738) is a rare primary immunodeficiency presenting with early childhood onset of recurrent life-threatening infections due to a lack of mature neutrophils. Nearly half of patients also display neurological symptoms, ranging from mild to severe developmental and cognitive delay often associated with epilepsy. Mutations in *HAX1* gene have been identified as the cause of KS. Notably, two transcripts of HAX1 exist (the shortest one missing part of exon 2) and only mutations affecting both of them are preferentially associated with the additional neurological phenotype. We report the third Italian patient with KS. DNA sequencing identified two novel compound heterozygous mutations: c.487C>T (p.Q163*) and c.557-2A>G. At age 22 years, our patient presents with a neuropsychiatric profile characterized by mild intellectual disability, reduced adaptive capacity associated to social anxiety and consequent impairment of social relationship. We focused on the neurobehavioral aspects of KS and recorded neurological symptoms in 26 additional patients from the literature. Comprehensive evaluation was available only for 16 of them, presenting with mild (38%), moderate (31%) and severe (31%) intellectual disability. Three patients showed a severe neurological phenotype associated with psychomotor regression. Seizures (primary or secondary generalized and usually responsive to antiepileptic drugs) were a common finding, being present in more than half of patients.

Motor clumsiness (19%), brain abnormalities (19%), and attention deficit (12%) were also found. This work expands the allelic spectrum of HAX1 and outlines high clinical variability of the neurobehavioral phenotype of KS.

J09.26

Schizophrenia as a genetic disorder: novel risk factors of neuroinflammation

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Introduction: Schizophrenia is a chronic incurable mental disorder. Despite several studies suggest a key role of genetic factors in disease development, molecular genetic markers of this disease are still undetermined. Here, we aimed to assess the role of genetic variations neuroinflammatory proteins in schizophrenia:
Materials and methods: For this purpose, a total of 500 chronic schizophrenia patients (ICD-10 code: F20.0, DSM-IV-TR code: 295.30) and healthy subjects unrelated Caucasian individuals of Armenian nationality were genotyped for the IL-6 rs1800795, TNF-a rs1800629, MCP-1 rs1024611, BDNF rs6265, NGF rs6330 and NGFR rs11466155, rs2072446 single nucleotide polymorphisms was performed using polymerase chain reaction with allele-specific primers (PCR-SSP).
Results: The distributions of genotypes for all selected SNPs both in patients and controls were in Hardy-Weinberg equilibrium. Our results showed that the rs1800795*C, rs1800629*A, rs1024611*G, rs6265*A, rs6330*T , and rs11466155*T, rs2072446*T alleles of the IL-6, TNF-α, MCP-1, BDNF, NGF, NGFR genes, respectively, are overrepresented in the group of patients compared to controls ($p<0.05$).
Conclusions: The findings obtained suggest that IL-6, TNF-a, MCP-1, BDNF, NGF, NGFR are among the candidate genes of schizophrenia and their rs1800795*C, rs1800629*A, rs1024611*G, rs6265*A, rs6330*T, rs11466155*T, rs2072446*T alleles might be nominated as risk factors for this disorders. This work was partly made possible by a research grant from the Armenian National Science and Education Fund (ANSEF#molbio-3125) based in New York, USA.

J09.27

Association study of 45 SNPs in Russian patients with schizophrenia.

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Although the clinical features of schizophrenia (SCZ) were described over one hundred years ago, our knowledge of the molecular and genetic mechanisms of pathophysiology remains very incomplete. The aim of this study was to analyze associations of 45 SNPs reported in GWAS with SCZ in Russian population of Siberian region. In this study 389 patients with SCZ and 674 healthy controls, matched to the patients by age, gender, and ethnicity were included. 15 SNPs were genotyped by real-time PCR using TagMan assay (Applied Biosystems) and 30 SNPs were genotyped by MALDI-TOF mass-spectrometry using MassARRAY Analyzer 4 (Sequenom). Allele-specific ORs and associated p values were calculated. We found six significant associations of SNPs with SCZ in Russian patients of Siberian region: rs12807809 at NRGN gene (OR = 0.55, p=0.000001), rs2247572 at KCNB2 gene (OR = 0.77, p= 0.03), rs2229741 at NRIP1 gene (OR = 0.81, p=0.02), rs11064768 at CCDC60 gene (OR = 1.79, p=0.001), rs16887244 at LSM1 gene (OR = 0.77, p=0.02) and rs7004633 at being between loci LOC100129100 and LOC100509857 (OR = 0.74, p= 0.01). These genetic markers were previously reported in GWAS associated with cognitive performance (rs2247572 KCNB2 gene, rs2229741 NRIP1 gene) and schizophrenia (rs12807809 NRGN gene, rs11064768 CCDC60 gene, rs16887244 at LSM1 gene, rs7004633 LOC100129100/LOC100509857). Genetic markers of NRGN, KCNB2, NRIP1, CCDC60, LSM1 and rs7004633 are associated with SCZ but their role in pathogenesis of the disease is not clear. Our findings also demonstrate that genetic variability in schizophrenia and cognitive performance has overlapping genetic background.

J09.28

Spinocerebellar ataxia type 28 Knockin mouse showed severe impairment of mitochondrial fission/fusion network in MEF cells.

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SCA28 is one of the 31 known subtypes of autosomal dominant Spinocerebellar Ataxias (SCA) and it is caused by mutations in the *AFG3L2* gene, encoding for m-AAA metalloprotease (mitochondrial ATPases Associated with a variety of cellular Activities).
We generated a knockin (KI) mouse model carrying the p.M665R mutation (corresponding to human p.M666R, showing the earliest onset in patients). Homozygous $Afg3l2^{M665R/M665R}$ mice were lethal perinatally; $Afg3l2^{M665R/+}$ heterozygous mice showed a significant motor impairment starting from 18 months of age. Number and dendritic structure of Purkinje cells, and the thickness of molecular and granular layers were almost identical in $Afg3l2^{M665R/+}$ and wild-type (WT) mice.
We studied mitochondrial dynamics in Mouse Embryonic Fibroblasts (MEFs) from KI and WT embryos: (i) we detected an increased amount of short OPA1 forms and the complete absence of the long bands in $Afg3l2^{M665R/M665R}$ MEFs; (ii) mitochondrial network morphology by mitoRED staining suggested a complete fragmented pathway in homozygous KI and an intermediate tubular/fragmented network in heterozygous MEFs; (iii) preliminary data revealed no differences in mitochondrial membrane potential in the three genotypes, despite a reduced ability of $Afg3l2^{M665R/M665R}$ MEFs in TMRM probe up-take, suggested by decreased basal fluorescence intensity. No others fission/fusion proteins was shown altered.
These data are in accordance with the cellular phenotype described in *Afg3l2* knockout mouse model, corroborating the idea that SCA28 mutations hitting the peptidase domain negatively impact on m-AAA complex function, probably acting as hypomorphic.
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J09.29

Association of mutations in 5´end of NF1 gene with a specific phenotype

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Autosomal domaminant disorder neurofibromatosis type 1 is one of the most frequent. It is causing by mutations in one of the biggest gene, in NF1 gene, consist of 60 exons. Gene product neurofibromin is a negative regulator of protooncogene Ras. Patients usually fulfill at least two of seven consensus clinical features: "café au lait" macules, freckling, Lish nodules, bone dysplasia, glioma of optical pathway, different types of neurofibromas and the first degree relative with confirmed NF1. In our cohort of 126 unrelated Slovak patients we identified 48/126 (38%) frameshift, 22/126 (17,5%) nonsense, 27/126 (21 %) splicing 16/126 (12,5%) missense mutations, 7/126 (5,5%) large deletions, 5/126 (4%) deletion of entire gene type I, and 2/126 (1,5%) small in frame deletions. Untypical splicing mutation we observed in 44,4% (12/27) patients. This mutations were identified deeper in intronic sequence or directly in exons as missense or nonsense mutations. We also followed position of mutations in NF1 gene. At 5´end which forms exons 1-16 (1-26 at new classification) we observed 43,8% (53/121) small mutations, by absence of mutations of entire gene. In patients with small mutation at 5´end we also observed significantly higher presence of Lish nodules, optical glioma and neurofibromas. This findings help us by desigining optimal diagnostic protocol and it confirms the advantage of using RNA based method. Complete mutation analysis in the first-degree relatives was performed in 61 families. Interestingly, we showed that 62% (38) of the patients from these families carry de novo NF1 mutation.

J09.30

Role of SCN2A, SCNB1, GABRG2 and PCDH19 in SCN1A-negative epileptic patients

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Introduction: Epilepsy is a large, phenotypically and genetically diverse group of disorders. Tens of different genes are linked to genetically determi-

ned forms. However, except for SCN1A, there is no other evident candidate locus with higher observed mutation frequency. In clinical practice, there is often an urgent demand for complete analysis of epileptic genes panel, as many patient express unclear phenotype. Therefore, there is need for genotype/phenotype correlation studies to search for associations, which can be used in diagnostic gene prioritization.
Materials and Methods: In 64 Slovak patients with a broad range of epilepsy phenotypes with no identified SCN1A mutation we sequenced the whole coding region and surrounding intron sequences of candidate genes SCN2A, SCN1B, GABRG2 and PCDH19 (27 females).
Results: In SCN2A, we have identified one causative variant (p.G211C) in patient with Lennox-Gastaut (LG) syndrome and one splicing-affecting nucleotide variant in GABRG2 (c.327+1G>A) in patient with complex partial seizures with generalization. No other disease-causing changes in selected genes were observed.
Conclusions: Since there were only two SCN2A and GABRG2 causative variants identified and neither SCN1B, nor PCDH19 causative variants were observed in any of 64 analyzed patients enrolled in the study, we can assume that there is no evident "gene hierarchy" in etiology of the disease.
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J09.31
rs1063843 polymorphism within CAMKK2 is associated with an increased risk of schizophrenia and bipolar disorder in Iranian population

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Background: A recent large-scale study have reported that rs1063843 located within the gene CAMKK2 is highly associated with schizophrenia in European and Han Chinese populations. Accumulating evidence shows that schizophrenia and bipolar disorder have common genetic variance. Here we evaluated the association of this variant with schizophrenia and bipolar disorder in Iranian population.
Methods: Genomic DNA was extracted from peripheral blood of Five hundred schizophrenic patients, 500 bipolar patients and 500 normal controls and all were genotyped for the rs1063843 using a PCR-RFLP method.
Results: The allele frequency of rs1063843 was significantly different in both schizophrenia and bipolar patients comparing to control group (P=<0.001, OR=1.4 and P=<0.00.1, OR=1.39 respectively).
Conclusion: For the first time we showed that rs1063843 is highly associated with bipolar disorder, although more replication studies are needed to confirm our findings. Our result also support the findings of previous studies suggesting an association between rs1063843 and schizophrenia.
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J09.32
Gene x environment interactions in personality: the role of ER-stress involved genes

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Personality traits are predictors of life outcomes as well as endophenotypes for major psychiatric disorders. Wolframin 1 (encoded by WFS1 gene located at 4p16.1 was previously linked to bipolar disorder) is involved in endoplasmic reticulum (ER) stress response. WFS1 gene polymorphisms were associated with psychiatric disorders and personality traits.
We aimed to examine gene-environment (GxE) and gene-gene (GxG) interaction models based on genes encoding proteins involved in ER stress: wolframin 1 (WFS1) and neighboring 4p16.1 region, activating transcription factor 6 (ATF6) and X-box binding protein 1 (XBP1) gene polymorphisms contributing into personality traits variation in healthy individuals.
In total, 1018 healthy individuals (68% women) from Russia (mean age±SD: 19.81±2.65 years) without any history of psychopathologies were subjected to personality traits assessment via TCI-125 and EPI. Involved individuals are Caucasians from Russian (N=409), Tatar (N=290), Bashkir (N=130) and Udmurt populations (N=189). Socio-demographic data including gender, order and season of birth, prenatal solar activity (SA), place of residence, childhood maltreatment were obtained. Genotyping of 48 SNPs was performed with SNPlexTM platform (Applied Biosystems). Statistical analysis was conducted with PLINK v.1.07 corrected via FDR-procedure for multiple comparisons.
The following GxE models have been revealed: ATF6 rs12045480*SA (PFDR=0.044) and WFS1 rs6828983*SA (PFDR=0.038) interactions affected neuroticism and Self-directedness, respectively. No significant GxG models were observed after FDR-correction. Accordingly, solar activity (measured as sunspots number) within gestation appears to modulate the effect of ER-stress related genes on personality. The possible mechanism of this modulation refers to epigenetic modifications occurred depending on high/low solar activity.
This work was supported by Russian foundation for humanities grant 13-06-00583a.

J09.33
Identification and functional characterisation of a novel dopamine beta hydroxylase gene variant associated with ADHD

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Dysregulation in neurotransmitter signalling has been implicated in the aetiology of ADHD. Polymorphisms of the gene encoding dopamine beta hydroxylase (DBH), a key player in catecholamine signalling, have been shown to be associated with increased risk for ADHD. Previous genetic studies of ADHD have reported associations with a range of DBH gene variants (rs2519152, rs1611115, rs1108580 and rs6271) however small sample sizes have led to inconsistency. Here we conducted TDT analysis in a large ADHD sample of 794 nuclear families to re-examine the relationship between DBH and ADHD. Although we did not replicate associations of rs2519152 and rs1611115 with ADHD, we identified a significant association with rs129882 (pcorrected = 0.02). Further, gene reporter assays of DBH rs129882 showed a significant impact of the ADHD-associated C allele on luciferase expression in a human neuroblastoma cell line, SH-SY5Y. These data demonstrate for the first time that a DBH gene variant which confers risk to ADHD is also associated with reduced in vitro gene expression.

J09.34
Functional genetic polymorphism of c-Jun in ischemic stroke

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Synaptic plasticity plays a key role in recovery of patients after progression and outcome of ischemic stroke. c-Jun is one of the most important transcription factors and is involved in regulation of transcription of genes involved in synaptic plasticity including formation of long-term memory. In present study we, for the first time, evaluated potential association of functional polymorphism of c-Jun encoding gene (JUN) with IS. For the purpose of study, genomic DNA samples of 125 patients with first episode acute IS and 153 healthy subjects (controls) with no family or past history of any mental, cerebrovascular or cardiovascular disorders were genotyped for JUN single nucleotide polymorphism (SNP) by polymerase chain reaction with sequence-specific primers (PCR-SSP).The primer sequences for the rs11688 polymorphism were as follows: forward 5′-TCCGCCTTGATCCGCTCC-3′ for standard allele, forward 5′- TCCGCCTTGATCCGCTCT-3′ for minor allele, and constant reverse 5′- AACCCAGGCGCGCTGAGC -3′. The results obtained demonstrated that the rs11688*T minor allele was significantly more frequent in controls than in patients (0.49 vs 0.31, P<0.0001, OR=2.6, 95% CI: 1.5-3.1), and the carriers of this allele were overrepresented in controls compared to patients (0.77 vs 0.51, P<0.0001, OR=3.3, 95% CI: 1.9-5.6). In summary, this study revealed that rs11688 functional polymorphism of JUN gene is negatively associated with IS and T minor allele of this locus may have a protecting effect against the development of IS.

J09.35

Pontine segmental cap dysplasia (PTCD) among five Egyptian patients suggests dominant de novo mutation

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Pontine tegmental cap dysplasia (PTCD) is a rare recently delineated mid-hind brain anomalies first described in 2007 by Barth and co-workers. It is characterized by a unique neuroimaging feature compromises the vaulted pontine tegmentum, which projects posteriorly into the fourth ventricle forming a "cap. It is associated with a variety of neurological features and cranial nerves affection likely a result of brain wiring defects. Skeletal, cardiac and gastro-intestinal malformations are variably associated. Herein, we describe 5 Egyptian patients, each from different families, presented MRI evidence of PTCD. Patients were all males and in 3 there was parental consanguinity, but no recurrence. All presented with hypotonia, cognitive and motor impairment and bilateral deafness. Corneal clouding due to anesthesia was in 3, which notably recurred in one after corneal transplantation. Other cranial neuropathies including trigeminal nerve affection with lack of pain sensation demonstrated clearly in one who lost his nasal collumella. Dysmorphic features in the form of preauricular tags, small ears and unilateral hypoplasia of mandibles was in one who had bilateral talipus. None of our probands had other systemic involvement and chromosomal studies were normal in all. This is the largest series from a single ethnic group with PTCD. We present clinical phenotype, compared previously described cases, aiming for more delineation of their clinical spectrum. The lack of recurrence suggests a *de novo* mutation in an unknown gene.

J09.36

Founder effect confirmation of c.241A>G mutation in L2HGDH gene and characterization of oxidative stress parameters in six Tunisian families with L-2hydroxyglutaric aciduria

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L-2-hydroxyglutaric aciduria (L2HGA) is an autosomal recessive neurometabolic disorder characterized essentially by the presence of elevated levels of L-2-hydroxyglutaric acid (LGA) in plasma, cerebrospinal fluid and urine. L2HGA is caused by a deficiency in the L2-Hydroxyglutaric dehydrogenase (L2HGDH) enzyme involved in the oxidation of LGA to the alpha 2-ketoglutarate. LGA has been proposed as an endo-and exogenous cytotoxic organic acid which induces free radical formation and generation of reactive oxygen species (ROS). In this report, we analyzed 14 L2HGA patients belonging to 6 unrelated consanguineous families originated from the south of Tunisia. The patients were diagnosed with L2HGA disease confirmed on the presence of high level of LGA in urine. We analyzed the L2HGDH gene in all probands and identified the same c.241A>G homozygous mutation. We also used intragenic SNPs and two extragenic microsatellites flanking the L2HGDH gene to confirm the founder effect of c.241A>G mutation in the fourteen studied cases. In addition, we carried out the measurement of the oxidative stress parameters in plasma of L2HGA patients which revealed a significant increase in the malondialdehyde levels (MDA), a biomarker of lipid peroxydation, and the reduced glutathione (GSH). A diminution of the antioxidant enzymes activities including superoxide dismutase (SOD), glutathione peroxidase (GPx) was also observed.

J09.37

A clinical variant in SCN1A inherited from a mosaic father cosegregates with a novel variant to cause Dravet syndrome in a consanguineous family

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Introduction: A consanguineous family from Turkey having two children with intellectual disability exhibiting myoclonic, febrile and other generalized seizures has been recruited to identify the genetic origin of this phenotype.
Materials and Methods: Physical and neurological examinations of all family members were performed and magnetic resonance imaging and electroencephalography recordings were analyzed with information on family history. A combined approach of SNP genotyping and exome sequencing were employed both to screen identified genes of Dravet syndrome and to detect homozygous variants common to affected sibs assuming a recessive inheritance model due to consanguinity in the family. The exome data analysis was extended further to potentially compound heterozygotes in the affected sibs.
Results: The study identified two paternally-inherited genetic variants in SCN1A (rs121917918; p.R101Q and p.I1576T), one of which was previously implicated in Dravet syndrome. Interestingly, the previously reported clinical variant (rs121917918; p.R101Q) displayed mosaicism in the blood and saliva of the father.
Conclusion: The study supported the genetic diagnosis of affected children as Dravet syndrome possibly due to the combined effect of one clinically associated (rs121917918; p.R101Q) and one novel (p.I1576T) variants in SCN1A gene. This finding is important given that heterozygous variants may be overlooked in standard exome scans of consanguineous families. Thus, we are presenting an interesting example, where the inheritance of the condition may be misinterpreted as recessive and identical by descent due to consanguinity and mosaicism in one of the parents.
This study was supported by TUBITAK (Project Number: 113S331).

J09.38

Determination of the Frequency of Spinocerebellar Ataxia (SCA) Types 1, 2, 3, 6 and 7 in northwest of Iran

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* Introduction: Spinocerebellar Ataxia(SCA) is an inherited disorder of brain function. It is characterized by increasing problems with coordination that often affect the legs, hands and speech. Since there is no information on the frequency of SCA in northwest Iran, the aim of the present study is to determine the frequency of common types of SCA disease (SCA1,2,3,6 and 7) and the number of CAG-trinucleotide repeats in the northwest iranian population.
* Material and Methods: Genomic DNA was extracted from the blood samples by salting-out. DNA samples were analyzed to determine the number of CAG trinucleotide repeats within each allele of the five causative genes. an abnormally large number of CAG repeats which is a diagnostic factor for the disease was detected by polymerase chain reaction(PCR) and agarose gel electrophoresis.
* Results: Of 118 cases,10, 8, 6, 8 and 3 cases were positive for SCA1,SCA2, SCA3, SCA6 and SCA7, Respectively. The range of the expanded CAG trinucleotide repeat was 39-75, 36-51, 50-84, 21-29 and 39-86 in SCA1, SCA2, SCA3, SCA6 and SCA7, Respectively.
* Conclusion: In this study for the first time in Northwest of Iran, the Frequency of common types of SCA(1, 2, 3, 6 and 7) was determined. Results show that the most common form was SCA1(8.4%) followed by SCA2(6.7%), SCA6(6.7%), SCA3(5%), SCA7(2.5%), respectively.
This study have been Sponsored by Ebnsina Genetic Laboratory of Tabriz,Iran

J10.01

Homozygous CCTG-repeat mutation in myotonic dystrophy type 2 in Russian patient.

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Myotonic dystrophy is a clinically and genetically heterogeneous autosomal dominant disorder. Myotonic dystrophy type 2 (DM2) is caused by unstable DNA sequences comprising CCTG-repeat in the first intron of the ZNF9 gene located at chromosome 3q21.
We present a rare case of DM2 with the expansion on both alleles of the ZNF9 gene.
The patient is a 72 year old man with mild DM2 phenotype. Onset of proximal arm weakness began at age 70 years (weakness during press-up), proximal leg weakness - at age 71 years (troubles with walking up the stairs). He has myalgia and hand stiffness, weakness of facial muscles, of deltoid, triceps and biceps brachii muscles (MRC 3/5), the elbow flexors (MRC 4/5),

elbow extensors (MRC 4/5), iliopsoas (MRC 3/5). The patient was operated on for immature cataract twice at age 67 and 68 years. He has no diabetes mellitus. A mild atrophy of muscles of gridle of superior extremities, of the quadriceps femoris are notable. EMG showed myotonic runs in limb muscles. Creatine kinase level is increasing since age 65 years and ranging between 600 and 1000 U/l. In addition, patient has sporadic episodes of arrhythmia, early balding. He has five clinically healthy children.
This is the first case of homozygous CCTG-repeat mutation in myotonic dystrophy type 2 described in Russian patients.

J10.02
A family with complicated hereditary spastic paraplegia-case study

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Hereditary spastic paraplegias comprise a group of rare neurodegenerative disorders characterized by slowly progressive muscle weakness, spasticity and hyperreflexia of the lower limbs. In different populations the reported incidences vary from 1.3 to 9/100 000 individuals. We present a 39 years-old male patient, born from healthy unrelated parents. Since the age of 19 he suffered progressive motor deficit of the lower limbs, initially with fatigue and irritability, evolving to paraparesis, spasticity, reduced muscle strength of upper train, neuropathic pain, back pain, bladder disorder, dysarthria. Neurological examination revealed marked spasticity with clonus and inability to maintain sitting position, muscle retraction, sensation of cold feet. Brain MRI was normal. Electromyography was also normal. The family history showed the existence of an affected brother, in whom the motor deficit was present only at 30 years of age, but of much lower intensity. The physical examination also revealed ataxia, moderate spasticity, orthostatism with support on the tip of the toes still possible, urinary urgency, normal sensitivity, obesity, mental retardation. The only other affected member of the family was a young woman, first degree cousin, with bilateral lower extremity spasticity, bilateral extensor plantar responses, hypertonic urinary bladder, onset of the symptoms was at the age of 23 years. They were considered to have complicated hereditary spastic paraplegia, with autosomal recessive inheritance. Clinical heterogeneity of the disease is revealed by variety and complexity of symptoms in the affected family members.

J10.03
Cognitive impairment and emotional instability-onset signs of isolated hypoplasia of corpus callosum

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Hypoplasia of the corpus callosum is a developmental disorder with complex biological basis. The prevalence was estimated at 2.05 per 10,000 live births in a study performed in southeastern Hungary. The precise incidence is difficult to establish, since many isolated cases are asymptomatic. It's etiology is complex, may be part of a syndrome or may occur isolated. We report a sporadic case with isolated hypoplasia of corpus callosum. The 26 years-old woman was born from healthy unrelated parents, after an uneventful pregnancy. The onset was insidious, six years ago, with cognitive disturbances, emotional lability, fatigue followed by progressive muscle weakness. Drug therapy and medical rehabilitation started immediately, but the evolution was rapidly progressive. She presented in our clinic complaining of motor deficit, hypoesthesia and paresthesia in lower limbs, cognitive impairment, concentration and emotional problems, anxiety, crying easily, dysarthria, posture and gait disturbances. Physical examination revealed body anteflexion, impaired balance and coordination, could not perform transfers from bed to chair or roll in bed, involuntary movements, muscle strength could not be assessed due to spasticity, spastic gait. Abdominal reflexes were abolished. Laboratory examinations were normal. Standard chromosomal analysis revealed normal karyotype. Brain MRI revealed isolated hypoplasia of corpus callosum. Spine MRI detected discrete medium dorsal spinal cord atrophy and intraspongious disk herniation D7-D9. The diagnosis was spastic tetraparesis due to hypoplasia of corpus callosum. The peculiarity of the case: although the defect was present at birth, she was diagnosed quite late, the only early symptoms were cognitive impairment and emotional problems.

J10.04
Homozygosity mapping data and exome data analysis of a family affected with a form of spastic paraplegia

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Hereditary Spastic Paraplegia is a heterogeneous group of inherited neurodegenerative diseases affecting the motor neurons, characterized clinically with the spasticity and weakness in the lower limbs. A family from Oman was identified with two affected boys to have an inherited form of Spastic Paraplegia. The parents are first cousins. The first child, born in January 2004, his parents noticed that he was developing normally till the age of six to seven months, when stiffness in lower limbs was observed. Overtime he was late in gaining gross motor milestones. There was weakness of both upper and lower limbs and severe spasticity of lower limbs, limiting his ability to sit and even walk. The other affected child, born in 2006, has similar symptoms which started developing around the same age of onset for his elder brother.
Homozygosity mapping is a versatile technique to investigate the genetic cause of autosomal recessive disorders. Children of consanguineous parents share regions identical by decent. To study the genetic basis of the disease in this family we analyzed SNP genotypes for the two affected family members and their parents generated by 330k SNP array from Illumina platform. None of the known SPG genes was identified using this approach. Exome sequencing data for the same family excluded all known SPG genes. Novel variants identified by exome sequencing in the homozygous regions are being investigated for genetic segregation in the family and for the possibility of being causative in this family.

J10.05
Neonatal detection in Sprintzen-Goldberg syndrome

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The syndrome of Shprintzen-Goldberg (SGS) is a little described entity (less than 60 cases reported), is suspected in individuals in the presence of craneosisnostosis, proptosis, retrognathia, micrognathia, cardiovascular malformations, marfanoid habitus, slight hypotonia to moderada, among others, rarely is it to diagnose neonatalmente.
Case: Our purpose is a male without product of second feat unprecedented importance for the current condition who from birth struck severe hypotonia, narrow suture, low implantation of ears, proptosis, ogival palate, retromicrognatia, arachnodactyly and marfanoid habitus. Methodology: The patient did not comply with the characteristics for Marfan syndrome and Loeys-Dietz syndrome, was the mutation bussqueda in TGFβ in peripheral blood and fibroblasts
Marfan síndrome (MFS) is a relatively common connective tissue disease with predominant involvement of the ocular, cardiovascular and musculoeskeletal systems. Experimental findings from mouse models of MFS have indicated that promiscuous latent TGFβ signaling. This group of MFS-related conditions includes Loeys-Dietz syndrome (LDS). Shprintzan- Goldber syndrome (SGS, aneurysms-osteoarthritis syndrome (AOS and syndromic TAA (sTAA). Common involvement of the TGFβ therapy may represent a general strategy to delay or even prevent dissection and rupture of the aortic wall in this group heritable disease.
The increasing recognition of exon-specific mutations in certain conditions, like Shprintzen-Goldberg syndrome, will likely allow the development of more efficient exon-targeted clinical diagnostic testing. Additionally, while exome sequencing is becoming increasingly valuable in the diagnosis or rare disorders, some genes such as SK1,are no well detected by existing technology. The current limitations of exome analysis and the continuing utility of Sanger-based sequencing

J10.06
Do ubiquitous herpesvirdae infections play any role in spinal muscular atrophy? Case series analysis.

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Spinal muscular atrophy (SMA) is a disorder caused by mutation in the *SMN1* gene, characterized by selective degeneration of lower motoneurons, and consecutively muscle weakness and atrophy. Despite simple and uniform mutation type, disease phenotype spans from neonatal death to ambulant cases with normal lifespan. Classification by types I, II, and III reflects this SMA's clinical polymorphism. *SMN2* copy number, *NAIP* mutation and several others are shown to be phenotype modifiers; however, phenotype correlation with these genetic factors is not strong.
In this, mainly cross-sectional, analysis we collected serological profiles (IgM, IgG) for most prevalent herpesviruses (HSV I/II, CMV, EBV) in patients of different ages and SMA types.
Results. Beside SMA type I infants (N=2) whose 100% abnormal serological

profiles and antibody origin require additional investigation, about 80% of type II (N=5) and 85 % of type III (N=7) patients have definite serological profile abnormalities characteristic for infectious state or, irrelevant to their age, clinical signs and/or epidemiologic history.
Conclusions. Very high proportion of abnormal serological profiles in all SMA groups may be relevant to phenotype severity. Herpesviruses are ubiquitous infections, which are capable of latent persistency and interaction by multiple pathways with motoneurons/myocites. Thus, detailed evaluation of SMA-viral interaction in longitudinal and experimental studies is warranted, because they could provide us with important insights of SMA pathogenesis.

J10.07
The role of immunohistochemistry and western blotting for dystrophinopathies management

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Introduction: Dystrophinopathies are a group of two recessive X-linked genetic diseases determined by gene mutation for dystrophin: Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD). The diagnosis for one of the two forms is based on immunohistochemical and western blot analysis of bioptic muscle tissue.
Matherial and methods: The study was performed in a family in which both boys have presented suggestive symptoms for dystrophinopathy. For a proper diagnosis, clinical and paraclinical examination, electromiography, have been completed with dystrophin genetic analysis, also immunohistochemistry and western blotting.
Results: The late onset and mild simptomatology innitially suggested a diagnosis of BMD but in time a very different pattern of symtoms have developed for the patients. Genetic analysis showed the same mutation for both brothers and their mother: 45-47 exon deletion, classically correlated with BMD. More severe clinical evolution for only one of the brothers raised the suspicion of a quantitative difference of dystrophin between the two cases. IHC and WB. have identified the complete absence of immunostaining for Dys 1 and 3 and a partial reduction for Dys 3 immunostaining in the case of the more affected brother, while the less affected, expressed only a diminished expression of Dys 2 and 3 in all muscle fibers and a reduction of Dys 1 immunostaining in some fibers.
Conclusions: Although genetic analysis is the only test which establish the diagnosis, based on genotip-fenotip international correlation studies between the two forms of the disease , analysis of dystrophin by IHC and WB turns into an important asset for prognosis and symptoms management.

J10.08
GJB1 gene mutation c.34G>A in a Lithuanian family

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Charcot-Marie-Tooth disease type 1X (CMT1X) is an X-linked dominant hereditary motor-sensory peripheral neuropathy, which results from mutations in the GJB1 gene and comprises approximately 10% of CMT cases. The GJB1 gene codes for connexin 32 (Cx32), a gap-junction (GJ) forming protein expressed by Schwann cells and oligodendrocytes. We report a Lithuanian family carrying the c.34G>A (p.Gly12Ser) mutation in the GJB1 gene. A 21-yr-old woman presented with distal weakness, unsteady walking, running difficulties and cognitive impairment. Physical examination revealed normal muscle strength, normal deep tendon reflexes and no apparent sensation dysfunction. Nerve conduction velocity (NCV) studies showed distal symmetrical sensorimotor axonal neuropathy with more prominent lesion in lower limbs. Brain MRI was not performed. Her father aged 56-yr-old complained of gait unsteadiness with bilateral hand weakness, muscle atrophy and contractures. Physical examination revealed tetraparesis with more prominent distal weakness (graded 2 score in distal muscles and 3-4 score in proximal muscles in both upper and lower limbs on the MRC scale) and distal muscle atrophy, severe sensory impairment in the pin-prick and vibration, sensitive ataxia, proximal hyporeflexia and distal areflexia. There were no cranial nerve disturbances, and no sign of dysautonomia. NCV studies showed severe distal symmetrical mixed type sensorimotor neuropathy. Heterozygous and hemizygous genotypes of the known c.34G>A (p.Gly12Ser) mutation (HGMD CM930318) were identified for daughter and father respectively in the GJB1 gene. Identified mutation is located in N-terminus cytoplasmic tail. This family report confirms that CMTX1 is a clinically heterogeneous group, with great variability in phenotypes, possible severe involvement in females with clinical features of cognitive impairment.

J10.09
Searching for mutations in the genes PARK2, PARK8, VPS35 in Slovak Parkinson's disease patients

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The Parkinson's disease (PD) is the second most common progressive neurodegenerative brain disorder caused by loss of nigrostriatal dopaminergic neurons, which affect the control of body movements, with formation of inclusions (Lewy bodies) in surviving neurons. Mutations in the PARK2 (parkin) and PARK8 (LRRK2) gene are those most frequently identified among patients with Parkinson's disease. The p.Asp620Asn (c.1858G>A) mutation in VPS35 was discovered as a new cause of PD in two independent exome sequencing studies. VPS35 encodes the vacuolar protein sorting 35 homolog, which is a part of the retromer complex involved in endosomal-lysosomal trafficking.
The aim of this study was to detect the prevalence of mutations within selected exons in LRRK2 (exons 24, 25 and 29), parkin (all exons except 2, 6, 7) and VPS35 (exon 15) genes in 146 Slovak patients with idiopathic Parkinson´s disease with both familial and sporadic forms of disease.
By sequencing the 15.exon of VPS35 gene we identified only a common synonymous single nucleotide polymorphism (rs168745, c.1938C>T, p.H646H). We did not find the c.1858G>A mutation in Slovak patients. In the selected exons of gene the LRKK2, we did not observe any mutations after sequencing. In parkin we found two coding polymorphisms and two probably pathogenic mutations.
In conclusion, the frequency of PARK2, PARK8, VPS35 (p.Asp620Asn) mutations in our Slovak population is low and does not play major role in pathogenesis of PD.
This study was supported by GUK/245/2015.

J10.10
Clinical study of patients with hereditary motor and sensory neuropathy from Republic Bashkortostan with novel mutation in GDAP1 gene.

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The Russian family with novel c.934G>A (p.Ala312Thr) mutation in the *GDAP1* gene displayed an autosomal-dominant pattern of inheritance. The onset of the disease was in the first or the second decade of the patient's life. The initial symptoms were pain, muscle weakness and wasting of distal extremities, gait disorder. The proband was a man 34 years old; his clinical picture was presented by slowly progressive moderate weakness and wasting of distal extremities, absent tendon reflexes, distal pan-modal sensory loss, bilateral *pes equinovarus* deformity. The proband's mother and sister had mild clinical symptoms of hereditary motor and sensory neuropathy: gait disorder, weakness and wasting of distal extremities, absent ankle reflexes. 11-year-old proband's daughter and 9-year-old nephew had complaints of pain in the calf muscles. Their clinical picture was presented by gait disorder and absent ankle reflexes. Neurophysiological data were available from the proband: median motor conduction velocities (MCV) were 35-38 m/s, the M-amplitude was 0.2-0.4 mV.
Thus, clinical and electrophysiological data of this family were presented by moderate or mild form of hereditary motor and sensory neuropathy with slowly progressive course of the disease.

J10.11
Correlation of copy number of the SMN2, SERF1A and NAIP genes with severity of spinal muscular atrophy in Serbian patients

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Introduction: Spinal muscular atrophy (SMA) is caused by deficiency of the SMN protein due to homozygous absence of the *SMN1* gene in 96% of patients. Despite genetic homogeneity, SMA is marked by extensive phenotypic variability, indicating the existence of disease modifiers. The *SMN1* gene re-

sides in the telomeric part of 5q13.3 segmental duplication enriched in genes and prone to various unequal rearrangements. Copy number polymorphism (CNP) of 5q13.3 centromeric counterpart of the *SMN1* gene, *SMN2*, is the main modifier of SMA phenotype, but the influence of rearrangements of other genes at 5q13.3 segmental duplication (*SERF1A* and *NAIP*) still remain elusive.
Materials and Methods: CNP of the 5q13.3 genes was determined in 99 Serbian patients with homozygous absence of *SMN1* (23 with severe type I, 37 with intermediate type II and 39 with mild type III), and 122 patients' parents by MLPA. Correlation of *SMN2*, *SERF1A* and *NAIP* CNP with disease severity, indicated by the type of SMA, was performed by Spearman rank test, while their joint effect was fitted using generalised linear models.
Results: Strong inverse correlation was observed between *SMN2*, *SERF1A* and *NAIP* CNP and SMA type (ρ=0.85, p=2.2e-16; ρ=0.659, p=6.661e-15; ρ=0.523, p=1.403e-08, respectively). Starting with full model including the *SMN2*, *SERF1A* and *NAIP* CNP and their interactions, the best minimal model describing SMA phenotypic variability included *SMN2* CNP (p<2e-16), *SERF1A* CNP (p<2e-16) and their interaction (p=0.02628).
Conclusion: Obtained results stress out that unequal rearrangements of 5q13.3 segmental duplication significantly contribute to phenotypic variabilityof SMA.

J10.12
effect of the VDR gene polymorphisms on susceptibility to Parkinson's disease in Iranian population

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Objectives: Parkinson's disease is one of the common and complex neurodegenerative disorders being affected by environmental and genetic factors. Vitamin D receptor (VDR) gene encodes a nuclear transcription factor that may play a role in neurological disorders. Recently, evidences in support of the effect of vitamin D receptor on development of PD have been increased significantly. In the present study, we investigated the association between three polymorphic sites related to VDR gene (Bsmi, G/A, Taq C/T, and Fok C/T) with the risk of the development of PD in Iranian population.
Methods: We analyzed 520 PD patients and 520 healthy control for three mentioned single nucleotide polymorphisms using polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP).
Results: Our results demonstrated that the BsmI and TaqI polymorphisms of the VDR gene among patients and healthy individuals had no significant differences in genotype and allele distributions. but we observed an association between the FokI C/T polymorphism and the Parkinson's disease (pValue= 0/001).
Conclusion: we showed that the FokI polymorphism of the VDR gene may have a role in the susceptibility to Parkinson's Disease in Iranian patients.

J10.13
HMSN-P is a new type of neuronopathy caused by mutation in TFG gene

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Introduction: Hereditary motor and sensory neuropathy with proximal predominance (HMSN-P) is considered a form of HMSN, so far identified only in families from the Far East.
Aims: Identify genetic cause of a neuromuscular disease in a large Iranian pedigree, delineate the disease, and describe presentations.
Methods: Linkage analysis and exome sequencing were performed. Thirteen individuals were studied. Two candidate sequence variations were tested for segregation with disease and screened in 420 controls. Subjective, biochemical, nerve conduction, electromyography, and muscle MRI data were obtained.
Results: A mutation in TFG was identified as cause of disease. The same mutation had been reported as cause of HMSN-P in patients of Far East ancestry. Phenotypic and genetic analysis revealed that the Iranian patients were also affected with HMSN-P. Three different haplotypes were recognized for the mutated alleles. The unreported or less common features of the Iranian patients include usual asymmetric initial presentation, involvement of abdominal muscles, and predominant paroxysmal dry coughing. Urinary dysfunction and rapid progression were sometimes observed. Hyperlipidemia was not common. Nerve conduction studies suggested sensory nerves are prominently affected. Muscle MRIs and clinical examinations revealed parallel involvement of both proximal and distal muscles.
Conclusions: HMSN-P, which is a misnomer, is not confined to the Far East and may have not been diagnosed in other populations. Absence of identity by descent in its only known mutation suggests at least three independent origins. There is clinical variability. Sensory nerves are prominently affected. Proximal and distal muscles are involved. The disease is a neuronopathy.

J10.14
Exceptional cases of choreic states in Russia: two cases report

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Chorae (choreic hyperkinesis) is the form of a hypermotility which is shown involuntary fast, chaotic, spasmodic, not stereotypic twitchings of various muscular groups. The reason of chorea's development are pathological changes of the corpus striatum. Character and localization of a pathological process are defined by features of a disease, degree of expressiveness and prevalence of diffusion and focal changes of a brain. One of exceptional cases with choreic hyperkinesis are choreoathetosis and congenital hypothyroidism with or without pulmonary dysfunction (CAHTP) and choreoacanthocytosis (CHAC).
CAHTP is an autosomal dominant disease with infancy onset of these clinical features, muscular hypotonia followed by the development of chorea, athetosis, dystonia, ataxia, and dysarthria. The reasons of CAHTP are mutations in NKX2-1 gene. We survey two patients of the age three months and one year with specific clinical features. New mutations c.344delG of NKX2-1 gene was revealed at the one-year-old boy. Normal sequence of NKX2-1 gene was detected for second patient.
CHAC is an autosomal recessive disease with red cell acanthocytosis, progressive neurodegeneration and onset in the third to fifth decade of life. The reasons of CHAC are mutations in VPS13A gene. We investigate 39-year-old man with acanthocytosis and neurologic abnormalities: progressive orofacial dyskinesia, dysarthria, dysphagia and choreic hyperkinesis of the trunk and limbs. Two new mutations c.85_86delCT and c.799C>T in compound heterozygous position were detected in VPS13A gene.
These two cases are first molecular confirmed cases of CAHTP and CHAC in Russia.

J10.15
Molecular genetic study of anoctaminopathy in a cohort of Russian LGMD patients.

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Limb girdle progressive muscular dystrophy (LGMD) are group of clinically and genetically heterogeneous polymorphic diseases characterized by primary lesion of pelvic and shoulder girdles.
LGMD 2L is an autosomal recessive slowly progressive disease characterized by late-onset proximal lower-limb weakness (mean onset age 35 years; range 11-50 years), asymmetric atrophy of the quadriceps femoris and subsequent atrophy of the biceps brachii. LGMD 2L development is determine by mutations in the anoctamin-5 gene (ANO5).
This form is the one of the most common autosomal recessive LGMD in Europe. It's prevalence in Finland is 2:100000, in northern England is 0.26:100000. We analyzed 295 DNA-samples in a cohort of Russian LGMD patients without mutations in CAPN3 and FKRP on the presence of frequent mutations in ANO5: c.191dupA (p.Asn64delinsLys) and c.2272C> T (p.Arg758Cys). The mutation c.2272C> T was found in 3 patients in heterozygous state and 1 patient in the homozygous state. The mutation c.191dupA wasn't found.
In the results of coding sequence assay in two patients with c.2272C>T mutation in heterozygous state other mutations weren't detected. It proposed that the second mutation is localized in non-coding and promoter sequences or it's an extended deletion / insertion. One patient have undescribed earlier nonsense-mutation p.Glu138Stop.
In analyzed samples the proportion of patients LGMD 2L was 1.4%.

J11.01
EP300 deletion: cause of Rubinstein-Taybi Syndrome 2

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We present a family, where deletion EP300 was diagnosed. Genetic counseling was recommended because of mental retardation and dysmorphic features in the boy. He was also operated because of posterior urethral valve and he was observed because of multiple renal stones. He had aortic valve stenosis and autoimmune thrombocytopenia. IQ score was 47. There

were dysmorphic features - hypertelorism, downslanted palpebral fissures, lower-set prominent ears, high palate, hands and feet were normal except hallux valgus.
We started genetic examination and we found normal karyotype 46,XY. Molecular genetic examination excluded X-Fragile syndrome, Prader-Willi-Angelman syndrome and Noonan syndrome. Microarray comparative genomic hybridisation (aCGH) identified a 1,5-Mb interstitial deletion of chr.22q13.1-q13.2, which included the EP300 gen connected with Rubinstein-Taybi syndrome 2. It was confirmed by fluorescent in situ hybridisation. Parents were examined and no mutation was found. Rubinstein-Taybi Syndrome is inherited in an autosomal dominant manner. Most of all mutations are de novo. Rubinstein-Taybi syndrome (RSTS) is a multiple congenital anomaly syndrome characterized by mental retardation, postnatal growth deficiency, microcephaly, usually with broad thumbs and halluces, and typical dysmorphic facial features with grimacing smile. About 50% of patients have RSTS1 due to mutation in the CREBBP gene and about 3% of patients have RSTS2 (OMIM # 613684) due to mutation in the EP300 gene. RSTS2 is usually associated with a milder phenotype than RSTS1. Our case was complicated because of normal skeletal findings with no classic malformations on both hands and feet in the boy. Grimacing smile was not so evident. Microarray comparative genomic hybridisation helped us identified the mutation and managed treatment/care.

J11.02
Cystathionine beta synthase mutations in patients with hyperhomocysteinemia and developmental and severe ocular problems

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Introduction: Three children from three different nuclear families had hyperhomocysteinemia, developmental delay, lens dislocation and glaucoma in the first decade of life. DNA sequence variations in methylene tetrahydrofolate reductase (*MTHFR*) and cystathionine beta synthase (*CBS*) have been reported to cause hyperhomocysteinemia-associated anomalies. *MTHFR* and *CBS* genes were therefore screened, to identify the genetic cause of hyperhomocysteinemia and ocular anomalies in these patients.
Materials and Methods: Hyperhomocysteinemia associated *MTHFR* polymorphisms C677T and A1298C were initially screened by restriction fragment length polymorphism method in the three families. *CBS* exonic sequences along with consensus splice sites were then Sanger sequenced. Mutation segregation in the parents and available family members was also confirmed through sequencing.
Results: *MTHFR* C677T and A1298C polymorphisms were found not to be associated with hyperhomocysteinemia, developmental delay and ocular phenotype. However, *CBS* sequencing resulted in identification of two novel mutations, a missense change (c.467T>C; p.Leu156Pro) in exon 7, an in-frame deletion (c.808_810del; p.Glu270del) in exon 10, and a recurrent missense mutation (c.770C>T; p.Thr257Met) in exon 10 of the *CBS* gene. The three different mutations in these three different patients were homozygous, which they had inherited from respective carrier parents.The identified mutations segregated with the phenotype in respective families.
Conclusions: This is the first report of *CBS* mutations from Pakistan where we identified two novel and a recurrent mutation. The persons carrying these mutations have developmental delay, early onset hyperhomocysteinemia along with severe ocular complications.

J11.03
Cohen syndrome identified by Next-Generation Sequencing (NGS) in an Iranian family

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Introduction: High throughput, large-scale parallel sequencing (next generation sequencing, NGS) technology has been installed in clinical laboratories for generate massive amount of sequence data from entire human genome in a short time with low cost as an ideal approach for the mutation analysis of hard-to-diagnose genetic diseases. This study presents an undiagnosed Iranian family with three affected members showing severe intellectual disability, distinctive facial features short philtrum, facial hypotonia, down slanting palpebral fissures and almond-shaped eyes, decreased visual acuity, narrow hands and feet and mild scoliosis.
Materials and Methods: Mutation analysis in one of affected patients was performed by NGS and validated by Sanger sequencing for confirmation in all three affected patients and parents.
Results: Exploration of obtained sequence data revealed new missense mutation in the homolog of the yeast vacuolar protein sorting 13 genes (VPS13B) as disease-causing mutation. So that all three patients shared the same homozygote genotype for this new mutation whereas parents was heterozygous.
Conclusions: In conclusion, since VPS13B have been reported as a cause of Cohen syndrome, this strategy was led to the clinical diagnosis of patients in this family with a phenotype strongly suggestive of Cohen syndrome. Thus, this study is a best example that demonstrated the incorporation of NGS and Sanger sequencing to identifying the etiology of such rare complex genetic diseases in short time.
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J11.04
2q21.1 deletion and 1q42.2 duplication in a girl with developmental delay, dysmorphic features and epilepsy, Further characterization of 2q21.1 deletion.

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The availability and use of CGH with high-resolution microarrays has greatly improved the detection of micro-deletion and duplications in patients with developmental delay and congenital anomalies.
Here we report the molecular karyotyping and phenotypic description of a new patient with 2q21.1 deletion and 1q42.2 duplication. Our patient presented with developmental delay, epilepsy, abnormal brain MRI and dysmorphic features. 2q21.1 deletion encompassing 28 genes (some of these genes are expressed in the brain) seems to be responsible for the clinical phenotype in the presented case. Duplications of 2q21.1 are rare with only a few cases reported to date in the literature. The phenotypic and genetic findings of our patient will be compared with those of previously reported individuals. We indicate the possible candidate genes, providing new data supporting further genotype-phenotype studies. Our results suggest that haploinsufficient genes within the deleted region, especially ARHGEF4 and GPR148, could underlie the developmental delay/intellectual impairment and epilepsy
Because of lack of the literature data, it is difficult to determine the clinical significance of the 1q42.2 (SIPA1L2, KIAA1383, NTPCR, PCNXL2 genes) duplication at this time.
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J11.05
10p15 deletion and 10p11-15 duplication in a patient with developmental delay and dysmorphic features

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Introduction
The multiple birth defects and mental retardation are associated with the unbalanced structural chromosomal rearrangement in proportion at 5%. The rate of deletions and duplications in all the chromosomal aberrations is 10%. We present here the patient who had unbalanced structural chromosomal rearrangement and dysmorphic feature.
Material and Methods
Cytogenetic analysis was performed on the patient's peripheral blood. Array CGH method was used for detection of deletion and duplication region.
Results
The 3-year-old female patient had motor mental retardation, dysmorphic feature, operated cleft lip, operated polydactyly, hearing loss and inability to walk and speak. Head circumference and length of the patient was <3p. She had dry hair, broad nasal root, telecanthus, upslanting palpebral fissures, micrognathia, high palate, crowded teeth, small low-set ears, joint hyperlaxity, hypotonia, dry skin and hirsutism.
It was reported that the patient was born in time with anoxia and cyanosis and hospitalized for 15 days due to sepsis. Her birth weight was 1900 gr. Her parents were not relatives. She was using drugs for congenital hypothyroi-

dism. The patient's karyotype analysis was 46,XX,add(10p13). Her parent's karyotype analysis were normal. The patient was thought to be de-novo. Array CGH was performed and it was detected that 10p15.3→15.1 region was deleted and 10p15.1→11.21 region was duplicated.
Conclusions
It was considered that 10p15 region was deleted and the region of 10p15 up to centromeres was duplicated , inverted and reinserted [inv dup (10) (p15.1- >p11.2)].
To our knowledge, this is the first and only report in which 10p15 deletion and 10p duplication occur together in a patient.

J11.06

Terminal deletion of 8p23.1 and terminal duplication of 8q22.1: San Luis Valley Syndrome with normal parental karyotype

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Recombinant chromosome 8 syndrome, San Luis Valley Syndrome, is characterized by duplication of 8q22.1-qter and deletion of 8pter-p23.1. It was first described by Fujimoto et. al in a Hispanic girl with multiple anomalies, including tetralogy of Fallot and minor anomalies due to the maternal pericentric inversion, inv(8)(p23.1q22.1). Characteristic features of rec(8) syndrome are congenital heart disease (conotruncal and septal defects), dysmorphic craniofacial features included hypertelorism and thin upper lip, anteverted nares, wide face, abnormally low-set ears, downturned mouth, low posterior hairline, micrognathia, brachycephaly, midface hypoplasia, and thick lower lip, developmental delay, mental retardation, and mild genitourinary tract malformations. Here we described a 3 year-old girl patient with developmental delay, facial dysmorhism, skeletal malformations, and unilateral hydronephrosis. Chromosome analysis from peripheral blood of the patient revealed a derivative chromosome 8. Parents had normal karyotype. Array CGH analysis detected 9.5 Mb deletion of 8p23.1-pter, and 47 Mb duplication of 8q22.1-qter. To the best of our knowledge this is the first report of the rec(8) syndrome with normal parental karyotype.

J11.07

Identification of a denovo novel mutation in FOXL2 gene in a Turkish patient with BPES

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Introduction: Blepharophimosis, ptosis, and epicanthus inversus syndrome (BPES) is a rare genetic syndrome which is characterised by eyelid malformation. Narrow palpebral fissures, ptosis, epicanthus inversus and telechantus are the main features of BPES. It is inherited in an autosomal dominant manner. In BPES Type I premature ovarian insufficiency is present with eyelid malformation. BPES Type II includes only eyelid malformation. Heterozygous mutations in FOXL2 gene which is located on chromsome 3q22.3 are responsible for both types of BPES. Chromosomal rearrangements involving this location also were repoted in BPES cases. In this study we report a case which was diagnosed as BPES and a novel mutation was detected .
Method: By evaluation of clinical findings and sanger sequencing of FOXL2 gene the case was diagnosed as BPES and discussed in the light of literature.
Case Report: A one year-old boy was referred us because of blepharophimosis. He had scaphocephaly, frontal bossing, ptosis, telechantus, narrow palpebral fissures, epicanthus inversus, flat nasal bridge, full cheeks, low set, big ears and clinodactyly of fifth toe. His parents' clinical examinations were normal. We sequenced FOXL2 gene and detected c.871_872insC variation. This variation was not reported previously, and seems to be resulting in an elongated protein. The variation was not detected in parents. Also in silico analysis of the variation was indicated that it is a damaging mutation.
Conclusion: Here we reported a novel denovo mutation in a BPES case. Fuctional studies and more reports are required to understand the affect of the mutation and for genotype-phenotype correlation.

J11.08

Alfi syndrome - cytogenetic study, FISH and clinical findings

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Alfi syndrome is a rare chromosomal anomaly that has an incidence of 1:50.000 newborns, but the incidence in Romania is unknown. We report a 5 year old girl with Alfi syndrome (a partial deletion 9p syndrome) a clinical entity as rare as it is well-defined. The clinical phenotype mainly consists of a distinct craniofacial dimorphism featuring, trigonocephaly, abnormal rotate ear, midfacial hypoplasia, arched eyebrows, down slanting palpebral fissures, small flat nose, anteverted nostrils, long philtrum, poor muscle tone and psychomotor retardation with language delay. A multidisciplinary evaluation was performed. It was observed that the patient presented female external genitalia, with agenesis of minor labia and no signs of virilization. The singularity of this case consists of the fact that the patient does not present hypothyroidism. Cytogenetic studies have been performed from peripheral lymphocytes by GTG banding in agreement with the standard procedure and ISCN 2013. A homogenous karyotype can be seen: 46,XX,del(9)(p22-pter). The anomaly is "de novo" because the normal karyotypes of the parents exclude balanced translocations. For the FISH testing probes have been used: Cytocell Aquarius Subtelomere Specific Probes: 9p (clone 43N6) - red (Marker STS: 9ptel30) and 9q (clone 112N13) - green (Marker STS: 9qtel33). The FISH probes have confirmed this deletion: 46,XX.ish del(9)(p22-pter)(43N6-,112N13+). In Romania, other 3 cases have been reported, but this is the first case to be documented using the FISH method. The early diagnosis of rare syndromes confers prognostic evaluation, counseling of the parents and better case management.

J11.09

A Case of Sotos Syndrome with a Novel Mutation of NSD1 Gene

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Sotos Syndrome or Cerebral Gigantism, is an overgrowth syndrome without endocrinological abnormalities. It is characterized by tall stature, advanced bone age, macrocephaly, prognathism, large hands and feet, neurological symptoms including mental retardation and characteristic facial signs. It is inherited in a autosomal dominant manner and is caused by heterozygous NSD1 gene mutations or deletions of 5q35 region including NSD1 gene. More than 95% of individuals have a de novo mutation.
We described a 5-year-old girl with Sotos Syndrome who has acromegalic symptoms, prognatism, strabismus, mental retardation and gait disturbance. DNA sequence analysis of the patient showed a de novo heterozygous c.5920 G>A (p.Glu1974Lys) mutation of NSD1 gene. This mutation is a novel mutation that has not been previously described. We have reported our patient to contribute to genotype-phenotype correlations in Sotos syndrome.

J11.10

Particular features in a cohort of Down Syndrome patients

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Aim: to present particular features associated in classic Down syndrome patients.
Material and methods: A 38 patients with Down Syndrome were diagnosed during a 5 year period, 49 % boys and 51 % girls. Six cases presented particular features. All patients performed complex investigations.
Results: From the selected 6 cases, one male infant was coming from a twin pair, both with Down syndrome, but the sister without heart malformation. All the 6 patients presented congenital heart disease: 3 cases with Fallot Tetralogy and 3 cases with atrioventricular septal defect(AVSD). The particular features in the Fallot Tetralogy were: in one case, association of aortic arch anomaly, with left subclavian artery emerging from left pulmonary artery, dolicocephaly, and cutis marmorata congenita, the second Fallot presented severe combined pectus carinatum and pectus escavatum and aneurysm of the pulmonary artery trunk at bifurcation, mimicking three branches emerging from the trunk; the third Fallot associated severe hypoplasia of the pulmonary trunk and branches, previous interventional dilated, but still waiting for stenting. The other 3 patients with AVSD, associated particular features as: congenital ectropion and cataracts in one case, grade IV right kidney hydonephrosis, neurogenic urinary bladder, right patellar and bilateral congenital hip luxation in another case and the third case associated cutis marmorata congenita, mimicking the shock.
Conclusions: particular heart and vascular malformations associated with the classic ones, cutis marmorata congenita, patellar and bilateral hip luxa-

tion, severe hydronephrosis, neurogenic urinary bladder, congenital ectropion and cataracts, all are particular features associated with Down syndrome, isolated described.

J11.11
Clinical and genetic characteristics Russian Marfan patients

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Marfan syndrome (MFS), a relatively common autosomal dominant hereditary disorder of connective tissue with prominent manifestations in the skeletal, ocular, and cardiovascular systems, is caused by mutations in the glycoprotein gene fibrillin-1 (FBN1). In MFS, there is a continuum of clinical presentations ranging from mild isolated features to severe progressive disease affecting multiple organ systems.
We herein present the results of the first direct DNA diagnosis in 9 Russian unrelated patients with MFS aged from 2 to 52 years. Eight 8 different heterozygous mutations in 8 probands was found (of which 6 were novel) and 1 patient had a substitution with unknown clinical significance described in SNP as rs112287730 with the incidence 0.02% (family analysis has shown its association with the disease in this family). Mutations were distributed along the length of the FBN1 gene. In contrast to the literature data, most of the mutations were null mutations: 3 frame shift, 3 splice site, 1 nonsense.
Most of the detected mutations (7 of 9) lead to the severe clinical presentation. Genotype-phenotype correlation analysis showed that mutations in exons 24-32 of FBN1 gene are associated with a high risk of a severe cardiovascular disease with a poor prognosis of life for all ages. Two patients with mutations in terminal exons 62 and 66 had severe changes in the skeletal, ocular, and cardiovascular systems (that diverges with the literature data).
Our results expand the spectrum of FBN1 mutations causing MFS and further confirm the role of FBN1 in the pathogenesis of MFS.

J11.12
Bilateral internal carotid artery agenesis associated with growth hormone deficiency, developmental delay and renal anomalies: a novel clinical entity?

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Internal carotid artery (ICA) agenesis is rare but is often syndromic and appears clinically heterogenous. It is associated with panhypopituitarism, velocardiofacial syndrome, Athabaskan-syndrome (AS), Bosley-Salih-Alorainy-Syndrome (BSAS) and PHACE syndrome. Here, we report a novel paediatric occurence of bilateral ICA agenesis.
A 4 year-old boy, born at term to a non-consanguineous British couple, was investigated for short stature (height: 82cm, -5SDS). He presented with facial dysmorphology (frontal bossing, hypertelorism, short nose, stellate bright blue irides and sclera), hypotonia and bilateral hearing impairment without any obvious focal neurology. He also has a dysplastic ectopic left kidney and developmental delay. Microarray analysis revealed a de-novo chromosome 11q14 microdeletion involving a single gene DLG2 encoding a synaptic protein. Pituitary function tests established isolated GHD (peak GH: 0.7mcg/L, IGF-1: 20ng/ml) and brain magnetic resonance (MR) imaging revealed a hypoplastic anterior pituitary and ectopic posterior pituitary. Bilateral agenesis of the ICAs with basilar artery ectasia was also confirmed by MR angiogram. CHARGE syndrome was excluded by analysis of the CHD7 gene. Other syndromes associated with ICA were considered unlikely on clinical grounds.
Hence, this case highlights a possible unidentified syndromic presentation of bilateral ICA agenesis. The loss of DLG-2 may account for developmental delay, but the basis of facial dysmorphology, renal dysplasia and visual abnormalities remain genetically unexplained. Homozygous HOXA1 mutations have been observed with ICA agenesis causing a phenotype overlapping BSAS and AS. However, its characteristic features (i.e. cardiac malformations, 6th nerve palsy and gaze abnormalities) are absent in our index case. Thus, further genetic investigation with whole exome sequencing may identify a genetic basis.

J11.13
Chromosome 4, Partial Trisomy 4q- case report

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Introduction:
Chromosome 4, Partial Trisomy 4q is a rare chromosomal disorder in which a portion of the fourth chromosome appears three times (trisomy) rather than twice in body cells . Associated symptoms and findings may vary from case to case. In most cases, the trisomy appears to result from a balanced chromosomal rearrangement in one of the parents; rarely, it is thought to arise from spontaneous (de novo) errors early in embryonic development occured for unknown reasons (sporadically).
Methods:
Case report of a 2 weeks old new born infant , with facial dysmorfism, swallowing disorders, 3rd left space parasternal intense ejection systolic murmur , generalized hypotonia.
From the anamnestic dates we retain: healthy 28 years old parents; gestational age 37 weeks, birth weight=2040g, Apgar score 9 at 1 minute, monitored pregnancy with oligoamnios.
Results:
The cardiologic consult and ultrasound revealed a congenital heard disease without cyanosis, congestive heart insufficiency type Ross III.
Karyotype - 46XXder(12)dup(4)(q31.2-qter)100%. Abnormal derivated chromosome identificated der(12). The abnormally chromosomial structure is a duplication of the distal region of the long arm of the 4th chromosome from the q31.2 until telomere. This unbalanced arrangement represents a partial trisomy (4)(q31.2-ter).
The evolution was unsatisfied and because of the swallowing disorders the child presented repeated respiratory problems, slowly upward weight curve and even bacterial endocarditis.
Conclusions:
Extremely rare disease in medical practice; investigated on the mother's side history, we discovered that both, mother and grandmother, have the same type of translocation, but only in this case we could make a correlation between the clinical manifestation and the chromosomal abnormality.

J11.14
Radio-ulnar synostosis, microcephaly and scoliosis in a 9 years boy - hallmarks of Giuffrè - Tsukahara syndrome

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In 1994 Giuffré et al reported a new genetic condition with microcephaly and radio-ulnar synostosis. Scoliosis, short stature and mental retardation were described, additionally, by Tsukahara (1995) and Udler (1998), in another syndrome. In 2005, clinical overlapping between those two conditions were suggested by Selicorni et al.
We report here a new case of a 9 years boy with short stature, mild facial dysmorphism consisting in microcephaly, proeminent eyes, flat malar region, thick uppper lip and micrognathia. He also had bilateral radio-ulnar synostosis, dorso-lumbar scoliosis, short stature (-2,24 SD) and mental retardation. These features oriented us to the Giuffré - Tsukahara syndrome. Less then 20 cases are reported in literature, most of them being familial. Our patient is a new case in the family. Bilateral cryptrochidism imposed a differential diagnosis with Noonan Syndrome. This particular phenotype brings additional evidence of the clinical overlapping beetween this two rare genetic conditions. The moleclar basis of the disease has not been identified.

J11.15
New mutation of the *TCOF1* gene in a patient with Treacher Collins syndrome

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Treacher Collins syndrome (TCS), is a well know syndrome first described in 1900. The estimated incidence is 1/50000 live births, with 60% of the cases resulting from a de novo mutation. It is characterised by a congenital disorder of craniofacial development with a combination of bilateral symmetrical oto-mandibular dysplasia and various head and neck defects. The characteristic facial dysmorphism includes bilateral and symmetrical hypoplasia of the malar bones and infra-orbital rim (80% of cases) and of the mandible (78%) (retrognathia, retrogenia). External ear malformation such as microtia or anotia, atresia of the external auditory canal and anomalies of the ossicular chain are often present (60%) with bilateral conductive hearing loss.

With an autossomic dominant transmission, the TCOF1 gene mutations are the most common cause of the disorder, accounting for 81 to 93% of all cases. POLR1C and POLR1D gene mutations are responsible for an additional 2% of cases.
The present work describes a new TCOF1 mutation in a 26 year old TCS patient, in a study done in order to provide prenatal diagnosis in a future pregnancy.
PCR amplification and DNA analysis through sequencing of all exons of TCOF1 gene and the contiguous intronic sequences were performed.
A new mutation c.2507del(p.Pro836Glnfs*37) in heterozygosity in TCOF1 gene was detected.
Every new information of a genetic condition should be reported in order to allow a more precise genetic counselling.

J11.16
Monitoring of congenital anomalies in the population of Republic of Moldova

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Introduction: Congenital anomalies (CA) took second place in the Republic of Moldova among the causes of child mortality. Regular monitoring of the CA is held since 1991. Relevant is the prevention of multiple congenital malformations (MCA) that require costly surgical treatment and characterized by a high mortality rate.
Materials and Methods: Was carried out the analysis of MCV in National Register of Congenital Anomalies of the Republic of Moldova for the period from 2009 to 2013.
Results: The prevalence of CA for concerned was 17.5 per 1000 newborns. The prevalence of MCA was 7.19 per 1000 newborns. Maximum prevalence of MCV was recorded in 2013, and minimal in 2012 (9.14 and 4.23 for 1000 newborns, respectively). MCV occupied the leading position in the overall structure of the CA (25,24±2,24%). During the reporting period, was mentioned the steady trend of increasing the prevalence of congenital anomalies from 19.8% in 2009 to 31.0% in 2013. The leading position occupied unclassified complexes of MCV - 47%, which manifested by congenital heart defects (CHD) with defects of musculoskeletal system, as well as CHD plus anomalies of the nervous system. Chromosomal syndromes (Down syndrome, Patau syndrome etc.) were in 38% of cases. Monogenic syndromes have been detected in 9% of children with MCV, and 6%of MCV were consequences of intrauterine infection and other diseases of the mother.
Conclusions. The above data of surveillance allow us to plan and carry out preventive measures to reduce the frequency of children's' birth with MCV in Moldova.

J11.17
A CASE WITH TETRASOMY 9p

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Introduction: Tetrasomy of short arm of chromosome 9 constitutes a clinically recognizable chromosomal syndrome.Tetrasomy of 9p is frequently caused by meiosis II nondisjunction followed by rearrangements leading to duplication of the short arm and loss of the long arm. Phenotypic abnormalities of tetrasomy 9p are ranging from mild developmental delay to multiple anomalies including intrauterine growth retardation, cerebral ventriculomegaly, dysmorphic facial features, cleft lip or palate, abnormal genitalia and renal anomalies. We present a new case of i(9p) that presented to us with dismorphic features such as hypertelorism, frontal bossing,corpus collosum agenesis.
Material Methods:Chromosomal analysis were performed on peripheral blood lymphocytes. Fluorescent in situ hybridization (FISH) studies were applied in order to confirm the origin of the extra chromosome.
Results: Chromosome analysis revealed tetrasomy 9p karyotype on GTL banding studies. Parental karyotypes were normal. Fluorescent in situ hybridization (FISH) studies confirmed the origin of the extra chromosome
Results: Here in, we diagnosed a case of tetrasomy 9p by classical cytogenetic methods combined with chromosome analysis and FISH.

J11.18
A further case with Macrocephaly-Capillary Malformation Syndrome

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Introduction: Macrocephaly-capillary malformation syndrome (M-CM, #602501) is characterized by a spectrum of anomalies including megalencephaly, prenatal overgrowth, brain or body asymmetry, cutaneous vascular malformations and distal limb anomalies such as polydactyly or syndactyly. The syndrome is frequently associated with structural brain abnormalities. Mosaicism of chromosomal abnormalities in skin biopsies and somatic mutations of PIK3CA gene was reported in M-CM cases in literature. In this study, we report a case with M-CM, diagnosed clinically and radiologically.
Materials and Methods: We present a case who was diagnosed as M-CM, and discuss the clinical and radiological findings.
Results: A 14 months old boy was referred to our clinic because of macrocephaly and Chiari 1 malformation. He had macrocephaly, prominent metopic ridge, hypertelorism, body asymmetry, capillary malformations on trunk, back of the neck and philtrum, and syndactyly of toes. Brain MRI revealed hydrocephaly and whole spinal MRI revealed Chiari 1 malformation and tethered cord. Peripheral blood karyotype analysis was normal, karyotype analysis from skin fibroblast culture was planned. Sequence analysis of PTEN gene revealed no mutations.
Conclusions: The case was diagnosed as M-CM according to clinical and radiological findings. Somatic mutations of PIK3CA gene are known to be responsible for M-CM syndrome, however, we could not perform molecular genetic diagnosis for this patient.

J11.19
A de novo 13.7 Mb interstitial deletion at 7q21.2 in a child with mental retardation, microcephaly, hearing loss and cerebral cavernous malformation

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7q21 microdeletion is a very rare chromosomal abnormalities characterized with mental retardation, short stature, microcephaly, and hearing loss. Additional features are split hand and foot anomalies and cerebral cavernous malformation. Her we described an eleven year-old male patient referred to the department of medical genetics because of microcephaly, sensorineural hearing loss, and facial dysmorphism. Cranial MRI revealed multiple cerebral cavernous hemangiomas and cortical dysplasia. Abdominal USG, echocardiography, cranial CT and X-Ray examinations were normal. Chromosome analysis of the patient from peripheral blood demonstrated an interstitial deletion at 7q21. Parental karyotypes were normal. Array CGH analysis showed 13.7 mb deletion at 7q21.2.

J11.20
Patau syndrome with mild phenotypic expression

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Patau syndrome is a genetic disorder which causes an infant to have three copies of genetic material of chromosome 13. Full trisomy 13 is caused by nondisjunction of chromosomes during meiosis. The extra genetic material disrupts the normal course of development, causing complex organ defects. Those individuals may have heart defects, polydactyly, intellectual disability, microphthalmia or anophthalmia, cleft lip, cleft palate, hypotonia, low-set ears, scalp defects, microcephaly, myelomeningocele, abnormalities of fingers, respiratory difficulcies and hypoglycemia.There is a possibility to stay alive, but most infants with the syndrome die within the first three months after birth.
Case report: We report 7 months old girl with Patau syndrome. She had low-set ears, flattened nose, wider then usual area between eyebrows, cataracts, coloboma and heart defects. Genetical tests were made two times during pregnancy, but higher risk for Patau syndrome was not shown . What is more fetus was tested with ultrasound but there were not found any abnormalities either. Trisomy 13 was confirmed by examining the infant's chromosomal pattern through karyotyping. Also it is important to highlight the fact that parents were in the middle age and healthy. Patient was treated with dorzolamide and timolum, dekstrose infusion, oxygen therapy and others. After all medication infant's condition was stabilized.

J11.21

Testicular Feminization or Androgen Insensitivity Syndrome (AIS), in Iran

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Background:The androgen insensitivity syndrome (AIS) or testicular feminization is a partial or complete inability of the cell respond to androgen. This syndrome is caused by inutility of androgen and so, testosterone converts into estrogen. The human androgen receptor (AR) is encoded by a gene located on the X chromosome. AIS occurs when the AR is impaired. This inexpressive to androgen hormone can damage or prevent masculinization of male genitalia in the developing status. AIS is divided in three categories, complete, partial and mild by intensity of external genital masculinization.
Methods:The specified screening was applied in 70 AIS patients and XY pattern was cytogenetically diagnosed and confirmed, out of 72000 families' medical records in Tehran Genetic Clinic. The essential basis to submit these patients to the Genetic Clinic from their gynecologist was primary amenorrhea. The other indicators are assessed such as: maternal and paternal age, parents consanguineous marriages, family history and clinical observation of the patients.
Results:The results specify that there was no association in maternal and paternal age. Marriage pattern of the parents has not shown any remarkable results in AIS cases. The clinical surveillance illustrated that amenorrhea has a high percentage in case study which may prove that it is the primary symptom of AIS patients.
Conclusion:The correlation of maternal and paternal age with AIS is not found. The parent's consanguineous marriage is high as a national culture in Iran. The frequency in clinical observation is reasonable and high percentage of this observation belongs to Amenorrhea. Therefore, amenorrhea is the most significant signs of AIS based on clinical assessments.

J11.22

Co-occurrence of supernumerary marker chromosome 22 and 17p13.3 microduplication in patient with developmental delay

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The delineation of karyotype-phenotype correlations in patients with chromosomal abnormalities is a central goal of clinical cytogenetics. However, the knowledge about structure of chromosomal aberrations strongly depends on the resolution level of molecular cytogenetic techniques. Here we report a 1.2 year old patient with severe developmental delay and a small acrocentric supernumerary marker chromosome 47,XY,+mar[13] according to conventional G-banding. Chromosomal microarray (60K, Agilent Technologies) revealed 22q11.1-q12.2 duplication (13,971 kb in size). Moreover, 733 kb interstinal 17p13.3 microduplication involving 11 genes (RPH3AL, LOC1000506388, C17ORF97, FAM101B, YPS53, FAM57A, GEMIN4, DBIL5P, GL004, RNMTL1, NXN) was also observed. The latter was mapped to the region of 17p13.3 microduplication syndrome. However the genes from critical region (YWHAE, PAFAH1B1, CRK) were not affected by CNV. The patient was born from the first pregnancy from healthy non-consanguinity parents. A boy presented a severe developmental delay with inability to hold up the head up to 7 months. He doesn't sit at the age of 1.2 years old. A clinical examination revealed microcephaly, downslanting palpebral fissures, epicanthus, hypertelorism, hypermetropy, astigmatism, divergent concomitant strabismus, small nose with saddle broad nasal bridge and flat tip, protruded low set ears, long smooth philtrum, short neck, hypertelorism of nipples. Early teething at the age of 4 month was noted. At the age of observation a boy has 10 teeth. Oval foramen of heart was also observed. Abdominal ultrasound revealed no pathology. Diffuse muscle hypotonia was noted. Our findings point out the significance of high-resolution array-based molecular karyotyping for patients with idiopathic developmental delay.

J11.23

20q Duplication Syndrome: A case report

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A 14 year old boy came to our clinic because of motor and intellectual disability. He had no prenatal history. His mother first took him to doctor when he was one and half year old because of walking problems. He had speech delay too. He had an operation of undescended testes and inguinal hernia. He has only left kidney. He has hyperthyroidism and glaucoma.His brain MRI and cardiac ecocardigraphy is normal. In his examination he looks like he is smiling. He has flapped-ears and exoftalmus. He doesn't have any other physical findings. First we made a chromosome analysis. Its result was 46,XY,der(20). The chromosome anlysis of his mother and his father were all normal. We decided to do an Array CGH with SNP Array-Illuminal HumanCytoSNP-12 BeadChip. Its result was there is a duplication of chr20:31.146.232-48 .340.036. Partial 20q duplication syndrome is rare. This report is one of the biggest isolated 20 q duplication.

J11.24

Case of Cri du Chat syndrome in Lithuanian health science university hospital

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Cri du Chat Syndrome (CdCS) or "cat-cry syndrome" is a rare genetic disorders resulting from a deletion of the short arm of chromosome 5 (5p-). Characteristic clinical manifestations of this syndrome are cat-like high-pitched cries, distinct facial dysmorphism, microcephaly and severe psychomotor and mental retardation. The size of the deletion ranges from the entire short arm to solely 5p15 and all include the CTNND2 gene. The prevalence is approximately 1 in 15,000 - 50,000 newborns and with females to male ratio of 4 : 3.
We report pacient, that was born at term (gestation age: 37 weeks) and was the first child of healthy, and unrelated parents. The family history was unremarkable. During pregnancy was diagnosed fetus hypotrophy and metabolic acidosis. After delivery, dextroposition of the aorta and a large subaortic ASD and VSD was detected by cardioechoscopy. When she was 6 months old, she had a heart defect corrective surgery and implanted cardiostimulators by reason of atrioventricular block. A girl was admitted for psychomotor retardation, also unable to sit and stand, incapable to chew and talk. Evident phenotypic changes: microcephaly, bilateral epicanthus, eye corners laterally slit down, long filter, triangle cranny mouth, narrow lips, high palate, micrognathia, hair on the neck, narrow feet and muscular hypotonia.
Cytogenetic analysis (FISH) revealed karyotype 46, XX, del (5) (p15) confirming diagnosis of Cri du Chat syndrome. This deletion was not find in girl parents, in fact this case occured as a de novo mutation. The reccurent risk for the parents was similar like in general population.

J11.25

C677T and A1298C mutations in methylenetetrahydrofolate reductase gene in patients with 21 trisomy and their mothers

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Introduction. Deficiency of methylenetetrahydrofolate reductase (MTHFR) causes a deficiency of the active metabolic form of folic acid, with important consequences, especially haematological (anemia, trombocytopenia) and hyperhomocysteinemia, a metabolic disorder which stimulates proliferation of smooth muscular cells, promoting early onset of atherogenesis, a risk factor for cardiac and cerebro-vascular disease. Folic acid deficiency is cited as promoting factor of chromosomal nondisjunction in gametogenesis, with increased risk for appearance of aneuploidy. The study aims to evaluate the prevalence of C677T and A1298C mutation in MTHFRgene in a group of children with 21 trisomy and in their mothers.
Patients and methods.The study group consisted of 73 patients with 21 trisomy and 67 mothers, who were registered in the Center of Genetic Pathology of First Paediatric Clinic Cluj in the period 2010-2014. Analysis of the two mutations was performed by PCR-RFLP technique.
Results. There were diagnosed 19 homozygotes (8 with C677T mutation and 11 with A1298C mutation), 16 composite heterozygotes and 31 heterozygotes (14 with C677T mutation and 17 with A1298C mutation). There were diagnosed 18 homozygous mothers (7 with C677T mutation and 11 with A1298C mutation), 15 composite heterozygotes and 28 heterozygotes (12 with C677T mutation and 16 with A1298C mutation).
Conclusions. Introduction of folic acid therapy in patients with 21 trisomy, homozygotes for C677T and A1298C mutations is beneficial in improvement of evolution of this patients, and treatment with folic acid in woman with homozygote or composite heterozygote genotype might reduce the risk of chromosomal nondisjunction in gametogenesis.

J11.26

Rare genetic syndromes confirmed in adult dysmorphic patients - clinical and molecular characteristics in 10 Slovak patients.

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Genetic counseling and DNA diagnostic tests in adult outpatients in our genetic clinic have been performed for various monogenic diseases. Genetic testing in adult patients with unexplained dysmorphy spans the smallest proportion, because majority of them was clarified in childhood. The aim of this study was to obtain cause and number of the adult patients undergoing genetic testing. We analyse results of genetic testing in group of 12 adults with various kraniofacial, skeletal, ectodermal dysmorphic signs. Clinical diagnosis have been made in childhood according to occurence of the major signs in 7 cases. This study offers survey of 10 different rare genetic syndrome and provide selected clinical and molecular data in Slovak patients. Causal mutation detected in Marfan, Hypochodroplasia, Ostechondrosis, Osteogenesis imperfecta, Albrigt, Oculodentodigital, Stickler and Cowden syndrome. Expanding spectrum of rare genetic syndromes which were confirmed at the molecular level during the last few years is fulfilling Eu initiatives on the issue of rare diseases.

J11.27

Familial osteoporosis and interstitial duplication of chromosome 5

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Osteoporosis is a systemic bone disease characterized by low bone mineral density and structural deterioration of bone tissue leading to an increased risk of fractures. Genetic factors have been recognized to play an important role in osteoporosis and a number of susceptibility genes have been identified and validated. Here, we describe an 11-year-old girl affected by severe osteoporosis with vertebral fracture and intellectual disability. Her father was affected by a severe form, too. Cytogenetic investigations revealed a dicentric chromosome 5, rearranged at the centromeric level, present also in her father. The two centromeres were both active, as demonstrated by immunological staining with CENP-C antibodies. Array-CGH of the proposita showed a large proximal duplication at 5p11q11.2 band and a small distal one at 5q11.2, while her father presented a unique duplication of 5p11q11.2 bands. The duplicated region on chromosome 5 contains ITGA1 (integrin alpha 1), an osteoporosis susceptibility gene, and FST (follistatin) gene. ITGA1 encodes integrin alpha 1 chain that binds to the beta chain to form a receptor involved in cell attachment and neurite outgrowth on laminin and collagen. Proper collagen-integrin interactions are important in fracture healing, which suggests that ITGA1 plays role in the regulation of mesenchymal stem cell and cartilage proliferation. FST encodes a monomeric glycoprotein that actively participates in the regulation of bone metabolism. Experiments performed in mice overexpressing follistatin showed a decreased quality of skeleton and susceptibility to bone fractures. In conclusion, interstitial 5p11q11.2duplication involving ITGA1 and FST could play a causative role in familial osteoporosis.

J11.28

The role of copy number variations (CNVs) in genetic mechanisms of 22q11.2 Deletion Syndrome

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The 22q11 chromosomal region contains low copy repeats sequences that mediate non-allelic homologous recombination, which predisposes to CNVs at this locus. Hemizygous deletions result in the 22q11.2 deletion syndrome (22q11.2 DS) that presents a highly variable phenotype, including congenital heart diseases. Most of cases present a ~3 Mb typical deletion, spanning LCRs A-D, and a minority of cases present a ~1.5 Mb nested proximal deletion spanning LCR22-A to LCR22-B; but other atypical CNVs have been reported. We used high-resolution array genomic hybridization (aGH) technique (CytoScan HD chip, Affymetrix®) to map the breakpoints at 22q11.2 region and investigate CNVs in other genomic regions in a cohort of 28 subjects with congenital heart disease and 22q11.2 deletion, previously detected by FISH and/or MLPA. Twenty-seven patients showed the typical deletion at 22q11.2 and one patient showed the proximal deletion. The size of deletion at 22q11.2 region varied from 1.8 Mb to 3.3 Mb. The breakpoints in 22q11.2 proximal region were from 18.644.790 to 18.916.842 kb and from 20.716.903 to 21.915.509 kb in distal region (Hg 19). The genes in these two breakpoint regions are variably hemizygous depending on the location of the breakpoints. 22 CNVs ≥ 300 kb outside the 22q11.2 region were detected, including a deletion at 11q14.3 (2.9 Mb) and duplications at 5q11.1 (628 kb), 6p21.2 (668 kb), 7p11.2 (662 kb) and 16q23.3 (367 kb). These regions could be potential loci acting as genetic modifiers contributing to the phenotypic variability found in the 22q11.2 DS.

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J11.29

Dominant mutations in KAT6A cause intellectual disability with recognizable syndromic features.

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The histone lysine (K) acetyltransferase KAT6A is prone to translocational events involved in acute myelogenous leukemia. Through a multi center collaboration study, we here report six individuals from five unrelated families, with mutations in KAT6A/MOZ detected by whole exome sequencing.
All five different de novo heterozygous truncating mutations were located in the C-terminal transactivation domain of KAT6A: NM_001099412.1: c.3116_3117delCT, p.(Ser1039*); c.3830_3831insTT, p.(Arg1278Serfs*17); c.3879dupA, p.(Glu1294Argfs*19); c.4108G>T p.(Glu1370*) and c.4292dupT, p.(Leu1431Phefs*8). An additional subject with a 0.23 MB microdeletion including the entire KAT6A reading frame was identified with genome wide array comparative genomic hybridization. Finally by detailed clinical characterization we provide evidence that heterozygous mutations in KAT6A cause a distinct intellectual disability syndrome. The common phenotype includes hypotonia, intellectual disability, early feeding and oromotor difficulties, microcephaly and/or craniosynostosis and cardiac defects in combination with subtle facial features such as bitemporal narrowing, broad nasal tip, thin upper lip, posteriorly rotated or low-set ears, and microretrognathia. The identification of human subjects complements previous work from mice and zebrafish where knockouts of Kat6a/kat6a lead to developmental defects.

J11.30

Discovery and genetic characterization of new neuropsychiatric syndromes from family-based studies in Utah.

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We conduct large-scale genomic studies of families in Utah, where there is a founder effect, large families, and good genealogical records, which enable well-powered genetic studies for rare diseases. We optimize exome and whole genome sequencing (WGS) to identify mutations that segregate with various syndromes, and we undertake comprehensive functional studies of identified mutations. We report the identification and characterization of many new genetic syndromes, including Ogden Syndrome, RBCK1 Syndrome, and most recently RykDax Syndrome. This latter syndrome presents with severe intellectual disability (ID) and very distinctive facial features. We show increased reliability of the biological inferences with an integrative WGS pipeline, including a new algorithm, Scalpel, developed for more accurate identification of indels. We find a 2 to 5-fold difference in the variants detected as being relevant for various disease models when using different sets of sequencing data and analysis pipelines, and we derive greater accuracy when more pipelines are used in conjunction with data encompassing a larger portion of the family. We have shown that 60X WGS depth of coverage from the Illumina HiSeq platform is needed to recover 95% of indels. We also developed SeqHBase, a big data-based toolset for analysing family-based sequencing data, and we demonstrated SeqHBase's high efficiency and scalability on several disorders, including with RykDax Syndrome, where we identified a maternally inherited missense variant in an X-chromosomal gene, TAF1. A "genotype-first" approach led us to other families with variants in TAF1 and a remarkably similar clinical presentation. More generally, we are setting standards for more accurate WGS, including for indel detection, in family-based studies.

J11.31

Clinical exome sequencing in Czech Kabuki-like syndrome patients

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Kabuki syndrome (KS) is a dominantly inherited multi-systemic disorder caused predominantly by de-novo mutations. Phenotypic features vary among KS patients and therefore it is difficult to diagnose this syndrome based on phenotype unequivocally. Currently, two disease genes have been associated with KS: *KMT2D* and *KDM6A*, but in 24-56 % of patients genetic basis of a Kabuki-like phenotype remains undetermined. In our previous study we examined KMT2D/KDM6A mutations in cohort of Czech patients referred for KS genetic testing. Seven "negative" patients were subjected to clinical exome sequencing (TruSightOne, Illumina, USA) in order to reveal pathogenic disease genes and variants responsible for Kabuki-like phenotype. In two male patients we found truncating mutations in EFTUD2 gene (linked to mandibulofacial dysostosis), in one of these patients in mocaics form, while another male patient had deleterious missense mutation in HUWE1 gene (X-linked mental retardation). Truncating mutation in KMT2C gene (previously linked to Kleefstra syndrome spectrum) was detected in female patient. KMT2C interacts with KDM6A and EHMT1 from this spectrum. In the remaining three patients potentially pathogenic missense and truncating variants in TGFBR2, KIF1A, RAI1, ILRAPL1, CDH15, SHANK3 and ZEB2 will be further assessed. We conclude that clinical exome analysis is a high yield method in Kabuki-like patients without mutations in KMT2D/KDM6A genes.

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J11.32

A girl with 2q36 chromosome deletion and phenotypic features of Waardenburg syndrome

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We report a 12 years old female patient with clinical presentation of MCA/MR syndrome including severe growth retardation, facial dysmorphism, microcephaly, limb anomalies, skin and hair pigmentation abnormalities, generalized hypertrichosis and mild mental retardation.

The child was born from a first pregnancy of young and healthy non-consanguineous parents with low birth weight (1600g), microcephaly and dysmorphic features noticed at birth. The karyotype of the patient was normal (46,XX). Because of the present severe growth retardation and microcephaly, right hand deformity, and generalized hypertrichosis with synophrys the initial clinical diagnosis was Cornelia de Lange syndrome.

The most prominent dysmorphic features at the age of 12 years included synophrys, hypertelorism and telecanthus, high nasal bridge, high arched palate together with generalized hypertrichosis and premature graying of the hair, skin hypopigmentation areas, and café au lait spots.

The performed array-CGH of the patient identified a heterozygous 6.759 Mb deletion of chromosome 2q36 (arr 2q36.1q36.3(222,508,616x2, 222,598,182-229,358,111x1, 229,409,228x2)) including 19 OMIM genes, with PAX3gene one of them.

Diseases associated with PAX3 gene include Waardenburg syndrome type 1 and 3. Waardenburg syndrome (WS) is autosomal dominant dysmorphic syndrome characterized by pigmentary abnormalities of the hair and eyes and congenital sensorineural hearing loss. WS is classified into 4 main phenotypes. WS type 1 is distinguished by the presence of dystopia canthorum. WS type 3 has dystopia canthorum and upper limb abnormalities, prominent nasal bridge and synophrys.

The detected 2q36 deletion explains the complex MCA/MR syndrome phenotype in our patient including symptoms of WS in association with other dysmorphic features.

J11.33

Partial trisomy 13q and partial 3pdel in a patient with a novel clinical finding due to paternal reciprocal 3p;13q translocation

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Individuals with 3p deletion present a great clinical variability. Apparently, a 1.5 Mb terminal deletion, including CRBN and CNTN4 genes, is sufficient to cause this syndrome. Partial trisomy 13q is an uncommon chromosomal abnormality with a variable phenotypic expression but in most cases, patients have a phenotype resembling complete trisomy 13. The aim of the present study is to describe a novel case of 3pdel/13qdup of chromosome 13 in a Mexican patient with a novel clinical finding. A 9 months male was the fourth child of non-related, young and healthy parents. There was no family history of congenital malformations. The child was born after 35 weeks of uneventful pregnancy. At birth, he had hepatoesplenomegaly, colesthasis and facial dysmorphism. On physical examination, he had a weight of 3800 (<3th centile), length of 62cm (<3th centile), upslanting palpebral fissures with long eyelashes, anteverted nares, high narrow palate, heart murmur, umbilic hernia and bilateral inguinal hernia with right cryptorchidism. Echocardiogram reported cardiac insufficiency with hypertrophic cardiomyopathy and pulmonar hypertension, ultrasound showed normal liver and brain CT revealed cortical atrophy. Conventional karyotype reported 46, XY, add(3)(p26). Karyotype from de father was 46, XY, t(3;13). Microarray assay exhibited an approximately 2.6Mb loss at terminal 3p26.3 and a 27.7Mb gain of the long arm of terminal chromosome 13 at q31.1q34. The presented case suffered a chromosomal unbalance with a partial trisomic component 13q31.1-q34 and a monosomic component 3p26.3 from paternal origin. Although clinical spectrum is too high in this chromosomal aberration, this type of cardiomyopathy has not been previously reported.

J11.34

Clinical and Molecular Characterization of Seven Egyptian Families with Autosomal Recessive Robinow Syndrome: Identification of Four Novel ROR2 Gene Mutations

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ABSTRACT

Robinow syndrome (RS) is a rare genetic disorder characterized by limb shortening, genital hypoplasia, and craniofacial/orodental abnormalities. The syndrome follows both autosomal dominant and recessive patterns of inheritance with similar phenotypic presentation and overlapping features. Autosomal recessive Robinow syndrome (ARRS) is caused by mutation in the ROR2 gene. Here, we present the clinical, radiological and molecular findings of eleven Egyptian patients from seven unrelated consanguineous families with clinical features of ARRS. Mutation analyses of ROR2 gene identified five pathogenic mutations distributed all over the gene. The identified mutations included four novel (G326A, D166H, S677F and R528Q) and one previously reported (Y192D). Our results extend the number of ROR2 mutations identified so far, suggest a founder effect in the Egyptian population, and emphasize the important role of genetic testing in proper counseling and patients' management.

J11.35

RHYNS syndrome is an MKS3/TMEM67-related ciliopathy

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RHYNS syndrome was defined as the association of Retinitis pigmentosa, HYpopituitarism, Nephronophthisis (NPHP), and mild Skeletal dysplasia. Few cases have been reported in the literature; yet, the underlying genetic bases are unknown. The occurrence in two brothers and the observation that only males are affected suggested either an X-linked or autosomal recessive inheritance. We revaluated a 38-year-old male patient presenting with retinitis pigmentosa leading to an extinguished electroretinogram by 11 years of age, NPHP since the age of 12 years, thyrotropin (TSH) and growth hormone (GH) deficiency, and mild skeletal anomalies including an abnormal pelvic configuration with irregular acetabular margins. He was one of the first patients described with this unusual pattern of association. Notably, there was no evidence of neurological involvement and he worked as a

computer technician. We used a panel of ciliopathy-related genes to unravel the molecular defect in this patient and identified biallelic mutations in the MKS3/TMEM67 gene. Mutations in MKS3/TMEM67 are associated to a vast range of ciliopathies encompassing Meckel syndrome (MKS) type 3, Joubert syndrome (JBTS) type 6, COACH syndrome, and NPHP with liver fibrosis (NPHP11). According to previously reported genotype-phenotype correlations, we identified one missense and one truncating mutation in MKS3/TMEM67, in a non-lethal phenotype. Our findings expand the clinical spectrum of MKS3/TMEM67-related disorders to include RHYNS syndrome.

J11.36
Mutations in *CENPF* cause apple peel intestinal atresia, microcephaly and ocular anomalies

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Apple peel intestinal atresia describes a condition characterised by a shortened small intestine with a coiled configuration around its vascular supply that resembles an apple peel. It often occurs as an isolated anomaly, but the association of apple peel intestinal atresia with other features, including microcephaly and ocular defects, has been reported, and is sometimes referred to as Stromme syndrome (MIM243605). Using exome sequencing we identified biallelic protein truncating variants in *CENPF* in a patient with apple peel intestinal atresia, microcephaly and ocular anomalies. The mutations were confirmed by Sanger sequencing and each unaffected parent was shown to be a heterozygous *CENPF* truncating mutation carrier. Neither mutation was present in the ICR1000 UK population exome series, nor the ExAC database of >60,000 exomes. Further sequencing of individuals with the same phenotype identified three additional cases from two unrelated families who also carry *CENPF* truncating mutations.
CENPF encodes a protein that is required for kinetochore function and chromosome segregation in mitosis. It is also involved in the regulation of DNA synthesis and hence cell cycle progression. Our findings demonstrate that the association of apple peel intestinal atresia, microcephaly and eye anomalies is a distinct recessive genetic syndrome caused by inactivating mutations in *CENPF*. We also demonstrate that exome sequencing provides a good strategy to identify causative genes in individuals with combinations of rare, distinctive clinical features. This work was funded by the Wellcome Trust Grant 100210/Z/12/Z.

J11.37
Clinical delineation of Schinzel alopecia-synostosis syndrome, a newly recognized condition of unknown genetic cause

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In 1980, Schinzel described a hitherto unreported multiple malformation syndrome in a 17-year-old girl featuring congenital alopecia, multiple synostoses of the upper limbs, joint contractures, cutaneous IV-V toe syndactyly, costo-vertebral segmentation defects, short stature and intellectual disability. Scrutiny of early literature identified two additional sporadic cases with overlapping features. Additional similar patients were subsequently reported by van Gelderen (1982), Dumić et al. (2000) and Schell-Apacik et al. (2008). Here, we describe a 4-year-old sporadic girl with growth delay of prenatal onset, micro-trigonocephaly, congenital alopecia and an unusual pattern of malformations showing striking similarities with Schinzel's original patient. In our patient SNP-array excluded chromosomal rearrangements at a resolution of 75 Kb, while sequencing of candidate genes including *RNU4ATAC, PCNT, MBTPS2, GJA1, PVLR1* and *CKAPL2* ruled out causative mutations. We had the opportunity to follow-up Schinzel's patient at the age of 53. She presented with severe short stature (126 cm), intellectual disability and reduced visual acuity due to retinitis pigmentosa-like changes. For years, the patient walked with support, but subsequently needed the use of a wheelchair. In an attempt to define the clinical characteristics of this condition, we compared data from published individuals and outlined a recognizable malformation pattern shared by our patients and that reported by van Gelderen (1982), thus delineating a unrecognized, likely rare alopecia-synostosis syndrome. The patients presented by Dumić et al. (2000) and Schell-Apacik et al. (2008) seemed affected by distinct disorders. Further molecular investigations are warranted to unravel the genetic bases of this condition.

J11.38
A new case of 8q21.11 microdeletion syndrome in a Mexican patient narrows the potential minimal critical region including the ZFHX4 gene

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The 8q21.11 microdeletion syndrome (OMIM: #614230) has been reported by Palomares et al in a series of 8 patients who presented a similar phenotype characterized by intellectual disability and common facial dysmorphic features that remarkably include round face, ptosis, wide nasal bridge, underdeveloped alae, short philtrum, cupid's bow of the upper lip, and downturned corners of the mouth. All patients comprised an overlapping loss of a 539.6kb that involves 3 genes: (1) the ZFHX4 gene which codes a transcription factor expressed in the adult human brain, skeletal muscle, and liver, (2) LOC10019232378 a miRNA with unknown function and (3) MRPL9P1 a pseudogene.
We report the case of a 4 year old Mexican female patient evaluated after presenting progressive hypotonia and global developmental delay accompanied with minor dysmorphic features sharing many of the characteristics previously described by Palomares et al. Array-CGH analysis was performed using probes for copy number and SNP on the Affymetrix CytoScan HD Platform with a resolution of 30kb for deletions and 60kb for duplications. The data were analyzed using the February 2009 NCBI human genome build 37.1(hg19) reporting a formula [hg19]8q21.11q21.13(77,751,515-83-,516,216)x1 indicating a microdeletion of 5.8Mb on chromosome 8q21.11-8q21.13 confirmed through FISH analysis using the probe (RP11-834L7).
The patient shares a deleted region of 14.7kb when compared with the previous series of 8 patients. Interestingly, this smaller overlap region includes the ZFHX4 gene narrowing the previously proposed minimal critical region for the development of the characteristic phenotype of intellectual disability and facial dysmorphic features.

J11.39
46,XX,dup(18)(q12.2q21.1) associated with encephalocele, periventricular nodular heterotopia, ostium secundum atrial septal defect and dysmorphisms: report of a case

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We present the case of a 16 months old child who came to attention to our clinical genetics unit for psychomotor retardation. Pregnancy was unremarkable until 31 weeks, when echography detected a parietal tumor, for which C-section was planned at 38+1 weeks. Born small for dates, the physical exam showed a 3x4 cm soft mass, covered by skin, on the parietal suture and a bone defect around the lesion. Craneal sonogram and MRI revealed an encephalocele with periventricular nodular heterotopia of the left ventricle, thinning of the corpus callosum and slight dilatation of ventricles bilaterally. No other malformations except for a ostium secundum atrial sept defect were present. She had surgery at 11 days, when the encephalocele was sucessfuly resected. Neurologic follow-up showed mild retardation of milestones. At 16 months she was not able to walk autonomously but no gross neurologic deficit was noted. Physical examination revealed short stature with normal head circumference and dysmorphisms: prominent forehead, thelecantus, short neck, slight malar hypoplasia, bilaterally short fingers with clinodactyly of the 5th and very short and proximally placed thumbs. Karyotype was performed and a 46,XX,dup(18)(q12.2q21.1) was detected. The duplication was characterized by array-CGH involving 17.6Mb and 68 genes. At 2 years of age the patient shows good evolution with no other medical complications. Similarities and differences with trisomy 18 and other segmental duplications of chromosome 18 are discussed.

J11.40
Exome sequencing reveals a mutation in Neuroblastoma Amplified Sequence gene in a family with early death, congenital fractures, dysmorphic Features and hepatic Failure.

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Background: Recent advances in molecular biology tools have enabled the identification of genes involved in rare disorders in families with small pe-

digrees. A Lebanese consanguineous family with 3 affected children was previously reported as presenting a newly individualized syndrome with neonatal spontaneous fractures, developmental delay, prominent eyes, generalised hirsutism, gum hypertrophy, and hepato-splenomegaly. Liver dysfunction was also observed and led to the early death of these patients.
Methods: To elucidate the genetic basis of this rare disease, a combination of genotyping and exome sequencing was performed.
Results: A homozygous missense mutation (NM_015909: c.C409T; p.R137W) was identified in the Neuroblastoma Amplified Sequence NBAS gene. The latter was already linked to a short stature syndrome with facial dysmorphism, optic nerve atrophy and leucocytes anomaly [MIM 614800]. A clinical comparison between both entities is discussed.
Conclusion: Here we report a newly described phenotype secondary to a NBAS mutation identified by exome sequencing in a Lebanese consanguineous family. Our paper expands the spectrum of disease linked to NBAS mutations and highlights the importance of exome sequencing in the delineation of the molecular basis of rare diseases especially when the clinical presentation is unclear.

J11.41

A *de novo* heterozygous *PLXDC2* variant is associated with a cerebellum-oculo-facio-skeletal syndrome.

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The Plexin Domain Containing Protein 2 gene (*PLXDC2*), located on the chromosome 10p12.31, encodes for a 529-aminoacid transmembrane protein expressed at high level in midbrain-hindbrain and cerebellum, and other tissues such as lung buds, heart, and spinal cord. In mouse model, functional analysis demonstrated that Plxdc2 acts as a mitogen for neural progenitors and as a receptor for Pigment Epithelium Derived Factor (PEDF), mediating many of its biological activities. Plxdc2 knock-out mice showed no obvious phenotype, whereas misexpression leads to severe alteration of neurogenesis during CNS development.
To date, any mutation in *PLXDC2* gene has been identified in human inherited disease.
Here we described a 6-year-old male patient showing a complex pattern of neurological and physical defects, including microcephaly, cerebellar atrophy, cataract, bilateral clubfeet, profound intellectual disability, and thorax deformity.
A trio-exome analysis disclosed a de novo missense variant in the *PLXDC2* gene. The variant is predicted to be deleterious from different bioinformatics tools, was not found in database of control individuals, and it leads to substitution of an evolutionally highly conserved aminoacid residue.
While the development of cellular and animal models will be necessary to prove the causality, we here discuss the potential role of the *PLXDC2* variant and the associated clinical condition, also with the aim to identify the potential phenotypes towards which extends the analysis of *PLXDC2*.

J11.42

Unlocking the 16p13.3 region by multiple molecular techniques: characterization of 14 novel deletions involving CREBBP in Rubinstein-Taybi patients

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Rubinstein-Taybi syndrome (RSTS, # 180849, # 613684) is a rare disorder characterised by cognitive impairment and congenital anomalies. Mutations in two homologous genes CREBBP and EP300 are responsible for ~55% and~8% of RSTS patients, respectively. CREBBP mutational spectrum includes point mutations (30-50%) and deletions (~10%). We report 14 novel CREBBP deletions identified by FISH and MLPA in a cohort of 171 RSTS patients. By FISH we detected five large deletions (from 480 kb to 1.35 Mb), one encompassing only CREBBP, whereas the others four extending beyond the gene at both the 5' and 3' ends. The patients displayed a phenotype often indiscernible from those carrying CREBBP point mutations, although a slightly more complex clinical presentation was observed. By MLPA we identified nine deletions (from 930 pb to 154 kb): two affect a single exon, the other seven remove one/ many CREBBP exon/s and a region including TRAP1 gene and a part of SLX4. Among these patients, the phenotype ranged across the spectrum found in point mutations carriers. All 14 deletions, were validated by aCGH, and their breakpoints precisely mapped.
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J11.43

G-banding study of peripheral blood cells in newborn infants with congenital malformations in north west of Iran

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Background: According to the world health organization, the rate of major malformations in newborn infants is about 3 million in each year. Chromosomal abnormalities are common and the most important causes of congenital anomalies. Conventional cytogenetic is one of the most important techniques to diagnosis of recurrent chromosomal anomalies. The aims of this study are determination of incidence and patterns of Chromosomal abnormalities in newborn infants with congenital defects.
Materials & Methods: This study was conducted during one-year period (2013-2014). The participants were recruited from medical genetic laboratory, Tabriz, East Azerbaijan Province, Iran. We analyzed 110 Peripheral Blood (PB) samples from 110 infants with congenital defects by G-banding.
Results: Among the 110 analyzed samples, 53 (48.18%) had normal karyotype. Chromosomal abnormalities were observed in 57 (51.82%) babies. Forty eight showed to have Down syndrome with observation as; 47, xy/xx +21 (89.58%), 46, xx/xy-i(21q) (8.33%), 46, xy+ t rob (13; 21)(q10; q10) (2.08%). Out of 9 remaining infants with Chromosomal abnormalities, one had mosaic karyotype as (46, xx/47, xx+13), 2 (47,xx+ 22 del q (13qter)), 2 (46, xy, 9q (del), 2 (47,xx+18), and 2 male infants with female karyotype.
Conclusion: According to this study in the north west of Iran, the rate of chromosomal abnormalities in infants with congenital defects is 48.18%. And the most common chromosomal abnormality is Down syndrome. These findings show that chromosomal abnormalities, particularly numerical of them are one of the most important causes of congenital anomalies in newborn infants. Therefore, conventional cytogenetic is an essential technique to consideration of newborns with congenital defects.

J12.01

The expression analysis of HMGA2 gene in uterine leiomyoma

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Background: Uterine leiomyomas, the most common gynecologic tumors in women, are attended scientifically due to their high prevalence, irrecoverable complications, and their high therapeutic burden. Genetic factors are among the factors playing an important role in the initiation and progression of these tumors. A subset of uterine leiomyomas, shows chromosomal rearrangements of the region 12q15, leading to an over-expression of the high-mobility group protein A2 gene (HMGA2). The purpose of this study was to assess the HMGA2 gene expression in samples of uterine myoma in Iranian patients.
Methods: The gene expression analysis was performed using quantitative RealTime PCR on 49 tissue samples including, 39 uterine leiomyomas and 10 normal uterine tissues as control. The relative quantification was assessed using REST software 2009.
Results: HMGA2 gene was significantly over-expressed in 89% of the uterine leiomyomas compared to normal tissue. However, the gene was not expressed in 11% of the leiomyomas.
Conclusion: HMGA2 gene over-expression plays an important role in the formation of the majority of tumors. Although it is not the only reason for the tumor development and the role of other genes should be concidered.

J12.02

Association between HIF and p53 in Endometrial cancer

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Background: Tumor suppressor p53, which shows many similarities to HIF-1 in terms of protein control by degradation, is predominantly involved in adaptation of cells to genotoxic stresses. p53 is a well-characterized transcription factor that plays a crucial role in responses to DNA damage, aberrant cell cycle control, apoptosis, and senescence. More than 60 target genes induced by HIF. In this study, we aimed to investigate the association between HIF-1 1772 C/T polymorphisms and polymorphism at codon 72 of Tp53 in endometrial cancer.
Materials and Methods: 75 patients with endometrial carcinoma and 75 patients whose underwent hysterectomy for non tumoral indication selected for evaluation of HIF-1 1772 C/T polymorphisms and mutations in exon 4 of the p53 gene by PCR-RFLP and sequencing.
Result: For the 1772 C/T polymorphism, the analysis showed that the T allele and genotype TT were significantly associated with endometrial cancer risk. In recent study, The rate of homozygote genotype of pro/pro or Arg/Arg in high grade group was higher than in comparison with low grade one. In addition samples that were undigested in RFLP, showed mutation in exone 4.
Conclusions: Our results suggest that the C1772T polymorphism of the HIF-1a and polymorphism at codon 72 of Tp53 may be associated with endometrial cancers. This report describes no mutual relations between p53 and HIF-1.

J12.03

hRgr overexpression in human T-cell malignancy

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Introduction: The expression of dtr-hRgr (diseased truncated human Ral GDS Related) protein produced by abnormal transcript of hRgr which is only observed in T-cell neoplasms, can induce cellular transformation through the activation of Ras and Ral GTPases. In this study, we purposed to determine eventual differences between hRgr and dtr-hRgr expression levels in subtypes of T cell malignancies initiatively. Thus we generate perspectives on inhibiting dtr-hRgr as a future treatment in T-cell ALL.
Materials and Methods: Peripheral blood samples were obtained from patients with T-ALL and B-ALL. Human T and B cell lines were used as controls. Total RNA was extracted from peripheral blood samples with using trizol methods. Gene specific oligonucleotides were used for the abnormal hRgr and human GAPDH in qRT-PCR. Relative fold changes were calculated using the 2-ΔΔCt method.
Results: qRT-PCR analysis was performed and overexpression of the abnormal hRgr transcript is only observed in T-ALL derived cell lines (Jurkat, CEM) and in human tissues with T-cell malignancies.
Conclusion: In with the aggressive treatment the prognosis for relapse in childhood T-cell ALL remains poor. In this study we indicate abnormal expression of dtr-hRgr in T-cell ALL. In ALL treatment, molecular tests are important for risk classification at initial diagnosis. dtr-hRgr may be a potential target for therapeutic approaches in future treatment in T-cell ALL.
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J12.04

Autophagy gene ATG16L1 in gastric cancer

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Gastric cancer remains a major global health burden, being the third leading cause of cancer death in both sexes worldwide. Recent evidence indicates the involvement of autophagy ATG16L1 gene in Helicobacter pylori infection and gastric carcinogenesis. Our study aimed to analyze the expression profile of ATG16L1 gene in gastric adenocarcinoma and to assess the possible association of ATG16L1 T300A (rs2241880) polymorphism with the risk of gastric cancer. A total of 350 Romanian subjects (108 patients with gastric adenocarcinoma and 242 healthy controls) were genotyped by allelic discrimination TaqMan-PCR assay with specific probes. ATG16L1 mRNA level was evaluated by qRT-PCR in biopsied tumoral and peritumoral tissue obtained by upper endoscopy from 34 patients. All genotype frequencies were distributed in accordance with Hardy-Weinberg equilibrium. A statistically significant association was observed for both AG and GG genotype carriers and furthermore, in a dominant model for carriers of G allele. Stratified analysis showed that the G allele of the ATG16L1 T300A polymorphism confers a protective effect, especially against non-cardia and intestinal type of gastric adenocarcinoma in the Romanian population. We found a tendency of increased expression of ATG16L1 in tumor samples when compared with the normal tissue. In conclusion, ATG16L1 T300A polymorphism may influence susceptibility to gastric cancer, mainly for the non-cardia and intestinal type in Eastern Europe. Our current research can improve the understanding of the autophagy pathway in gastric carcinogenesis.
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J12.05

Frequency of co-polysomy of chromosome1p and 19q in oligoastrocytomas

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Gliomas are the most frequent primary brain tumor of adults. Gliomas with oligodendroglial component (oligodendrogliomas) are relatively rare and associated with longer survival than astrocytic gliomas (astrocytomas). Cellular and morphological distinction between oligodendroglioma and astrocytoma can be subjective with inter-observer variability. It is well established the impact of co-deletion of chromosomes 1p36 and 19q13 in oligodenrogliomas. There is also a high incidence of 1p and 19q deletion in oligoastrocytic glial tumors (oligoastrocytomas). Co-deletion of 1p/19q is associated with a positive prognosis and survival. These deletions occurred in 60-70% of oligodendrogliomas and they are less common astrocytomas (about10-20 %). Based on our literature search and best knowledge, the frequency and impact of polysomy status for given chromosomes have not been deeply investigated.
In this study we have evaluated the polysomy status of 1p/19q in 45 paraffin embedded tissue of oligoastrocytomas by using florescent in-situ hybridization (FISH). Tumors were 33 oligoastrocytoma (OAs) WHO Grade II, 11 oligoastrocytoma WHO Grade III, and 1 glioblastoma multiforme with oligodendroglial component (GBM-O). We found 12 co-polysomy of chromosome 1p/19q in our series (26,66 %). 8 were belong to OAs WHO grade II (24,24 %) and 4 were belong to OAs WHO grade III (36,4 %).
However previous studies suggested that co-polysomy of chromosome 1p/19q has worse prognosis in oligodendrogliomas, there is no detailed information for astrocytoma or oligoastrocytomas. All of our cases were newly diagnosed therefore we don't have long-term follow-up data.

J12.06

Karyotypic diversity in a patient with chronic myeloid leukemia treated with tyrosine kinase inhibitors and development of secondary acute myeloid leukemia

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Case report: We present a patient with Philadelphia-positive (Ph+) chronic myeloid leukemia (CML) who developed a resistance to therapy with imatinib mesylate which coincided with the appearance of t(5;6;12) in the Ph+ cells. Despite karyotype evolution, the patient remained in a chronic phase of CML and mutation analysis of kinase domain was negative. She was switched to Nilotinib, and her response fluctuated during the time. With continuation of nilotinib and its escalation to 800mg, she achieved major cytogenetic response (MCR) at 18 months (10% of Ph+ clone with t(5;6;12)), and complete cytogenetic response (CCR) after 24 months. Her further follow up showed stable CCR and MR3 for another three years. During this period, a Philadelphia negative (Ph-) clone with +8 appeared and persisted one year. After its disappearance, the patient developed neutropenia, without signs of anemia and thrombocytopenia but with elevated transaminases. Immediate bone marrow evaluation revealed dysplastic changes and 6% of blasts. Karyotype analysis revealed poor chromosome morphology with monosomy in C-group. By interphase fluorescence in situ hybridization (iFISH), monosomy 7 was detected. At the same time, the patient was negative for Ph chromosome and BCR/ABL fusion by both cytogenetic and iFISH analyses and was still in MR3. In another 6 months, she developed acute myeloid leu-

kemia and shortly after induction therapy, died.
Conclusion: The present case suggests the importance of the morphologic and karyotype follow-up of patients on TKI therapy even after achieving CCR.

J12.07

Nested Methylation Specific PCR for MGMT promoter methylation test in prediction of radiotherapy and alkylating agents based chemotherapy of Ewing sarcoma tumor

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Introduction: MGMT (O6-methyl-guanine-DNA methyltransferase) is a protein with a specific enzyme activity that is involved in DNA repair. MGMT enzyme repairs DNA alkylation damage, introduced by classical chemotherapy, and also the double strand braking points introduced during radiotherapy. Epigenetic inactivation by promoter methylation of the MGMT gene is very well established. This gene is epigenetically silenced in a variety of cancers, especially glioblastomas, colon cancer, non-small cell lung cancer, gastric carcinoma, head and neck squamous cell carcinoma and also in Ewing sarcoma tumors.
Materials and methods. Ewing sarcoma tumors (2 specimens) were collected from diagnosed patients. Methylation specific (MS) PCR and Methylation specific MLPA methods were used for estimation of the MGMT transcription state through its promoter methylation pattern. Neste MSPCR was used instead of classical MSPCR in order to increase the sensibility and specificity of the method.
Results: The nested MSPCR gave better and clear results as compared with classical and MSMLPA methods. Certain specific conditions for optimization are described.
The Nested MSPCR method proved its efficiency in characterizing the methylation pattern of MGMT gene. The clinical significance of this estimation is linked with the repair capacity of the MGMT enzyme of the tumour damage introduced by classical (alkylating) chemotherapy and radiotherapy. The resistance of Ewing sarcoma tumors was correlated with the MGMT activity and nonmethylated state of its coding gene promoter.

J12.08

Diagnostic panel for testing of germline mutations associated with breast and/or ovarian cancer in Russian population.

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Genetically based breast and / or ovarian cancer is one of the most common forms of family
malignant tumors. Germline mutations in breast cancer are found in 10%, in ovarian cancer in 15% of cases. The routine biochip diagnostic for founder mutations in Russian Federation (185delAG, 300T> G, 4153delA, 4158A> G, 5382insC in BRCA1, 6174delT in BRCA2, 1100delC in CHEK) is commonly used. Additionally, the association of other mutations in NBS1, BLM, KRAS, TP53, PALB2 gene with the higher risk of BC/OC familial cancer has been found in the Slavic population. Also, many researchers have described new clinically relevant mutations in genes BRCA1, BRCA2, CHEK2. To optimize the genetic testing an improved diagnostic panel has been established, including also the following mutations: 2073delA, 3819delGTAAA, 3875delGTCT (BRCA1), 470T> C, IVS2 + 1G> A (CHEK2), R72P, IVS6 + 62G / A (TP53), rs61764370 (KRAS), 172_175delTTGT (PALB2), Q548X (BLM) and 657del5 (NBS1). Analysis of DNA samples of BC/OC patients in has shown the importance of these genetic markers for the Russian population. In addition, searching for new mutations BRCA1/BRCA2 in patients with familial form of BC/OC cancer was performed using the 454 technology (Roche). BRCA1/BRCA2 coding regions were amplified using the BRCA MASTR v2.1 Assay (Multiplicom). Two rare pathogenic mutations (rs80357433, rs80357123) were found in patients with bilateral BC. c.4689C>G mutation (rs80357433) forms a premature stop codon in the BRCA1 gene. The mutations were confirmed by Sanger sequencing. Further investigations using NGS may help in searching of new pathogenic mutations in Russian population.

J12.09

Investigation of the melatonin effect on apoptosis and differentiation in breast cancer stem cells

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Introduction: Cancer is still one of the major health problems. Even though there are significant developments on the anti-cancer treatment, but 5-year survival rates were not significantly improved. In literature, it has been discussed that the main failure of treatment was related to cancer stem cell (CSC) behaviours due to many challenges in the targeted therapies. Melatonin and like chemicals have been used for different diseases. Some studies showed that melatonin is affective on cancer in different ways. However, there is no data about the effect of melatonin on CSCs in the literature. In the present study, we aimed to investigate the effect of melatonin on apoptosis and differentiation in breast CSCs *in vitro*.
Materials and methods: After appropriate treatment time and dose (IC50) were determined by using MTT, MCF7 breast cancer and HEK293 control cells were treated with melatonin. The effects of melatonin on apoptosis, number of CSCs and differentiation were evaluated by flow-cytometry with Annexin-V, CD44+/CD24- markers and MTT, respectively. In order to confirm the FACS results, apoptotic pathway target *BAX* ve *BCL-2* and CSC marker genes (*SOX2*, *NANOG* and *OCT4*) were analysed by using qPCR.
Results and conclusion: Melatonin increases apoptosis and differentiation in the MCF7 CSCs, but it decreases both in control. This result indicates that the effect of melatonin needs to be studied on the other pathways in order to clarify the therapeutic effect *in vitro* and *in vivo* experiments.
This study was supported by TUBITAK project # 113S478
Key words: Breast cancer, cancer stem cell, melatonin, apoptosis, differentiation

J12.10

„Evaluation of BIOMED-2 Molecular Gene Rearrangement Protocols for Clinicopathological Diagnosis of Hodgkin Lymphoma"

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Analysis of molecular clonality was performed by applying BIOMED-2 protocols to evaluate immunoglobulin gene rearrangements patterns in Hodgkin's Lymphoma (HL) cases). We implemented a standard protocol in HL cases, which have been previously suggestion for clonality detection on formalin fixed, paraffin-embedded (FFPE) tissue of non-Hodgkin lymphoma (NHL) patients.
We investigated 50 consecutive FFPE specimens of HL cases, which consisted of 43 cases of cHL and 7 cases of nodular lymphocyte-predominant¬¬ Hodgkin's lymphoma (NLPHL). Positive CD30, CD20, CD15, CD3, LCA and Facsin markers and IGH, IGK, IGH D-J, and IGL monoclonality in cancerous cells were evaluated using immunophenotyping, BIOMED-2 protocols and Heteroduplex analysis method.
Overall, our finding showed 94% (47/50) clear rearrangements in HL cases; consisting of 74% (37/50) in IGH, 70% (35/50) in IGK, 42% (21/50) in IGH D-J and 44% (22/50) in IGL. IGH clonality detection has related to positive CD30, CD15 ($P<0.005$) as well as LCA and Facsin population cells ($P<0.005$). In addition, the relationship between IGK clonality and CD20, CD3-positive population cells ($P<0.005$) were seen as statistically significant.
Analysis of clonal gene rearrangements in IGH and IGK genes using BIOMED-2 protocols could be implemented as a valuable method for increasing sensitivity (94%) and accuracy of HL similarly to NHL.

J12.11

Clinicopathological and genetic characteristics of breast cancer in Bulgarian women

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One in 14 women in Bulgaria will develop breast cancer (BC) during her life (age 75). Despite the advantages in therapy worldwide, prophylactic measurements are the only certain way of handle with this disorder. One of the well-recognized management tool is genetic screening for the main BC susceptibility genes (BRCA1 and 2). There are more than 3000 distinct

mutations in both genes and alterations are two types - point mutations and large genomic rearrangements (LGR).
Each population has specific clinical, histopathological and genetic characteristics of BC. The aim of our study was to investigate them among Bulgarian women with BC.
The total number of patients included in the study (recorded in the Cancer Registry of University Hospital, Pleven) was 176 women with BC, with average age at diagnosis 57 years. On the basis of preliminary selected criteria, 80 women (with average age at diagnosis 50 years) were referred for genetic testing of BRCA1 and BRCA2.
All of them (100 %) were screened for deleterious point mutations - two in BRCA1 (C61G, 5382insC) and three in BRCA2 (6079del4, 9326insA, 9908insA) and 89% for LGR.
The results of our study were: 20 (11%) of women showed familial BC, 21(13%) of them - early onset BC, 20 (13%) had TNBC and only four of them (2%) had bilateral BC. We found only one point mutation among selected patients - 5382insC in BRCA1 in 2, 5% and did not find LGR.
The established data were similar to the reported data for other European populations.

J12.12
Detection of Immunoglobulin IGH Gene Rearrangements on Formalin-Fixed, Paraffin Embedded Tissue in Lymphoid Malignancies

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Human lymphomas are aggressive malignant diseases, which can be categorized based on their B and T cell lineage. B-cell lymphomas form around 90% of the total lymphoma cases, whilst the remnants of malignancies arise from the T cell branch. Lymphomas are mostly characterized as clonal proliferations of specific tumor cells. The detection of malignant lymphomas are extensively investigated by their morphological features, immunohistochemistry and flowcytometric immunophenotyping, but in some cases remain unknown. The BIOMED-2 protocols were used to determine the clonality of IGH gene rearrangements in patients with lymphoma. PCR amplification was performed on FFPE of 50 patients with B-cell lymphoma, which consisted of 11 cases with HLs, 25 cases of B-NHLs and 14 cases of B-LPD (lymphoproliferative disorders) which were diagnosed as unclassifiable lymphoma. The rate of positive clonality was detected in 96% (24/25) of B-NHLs, whereas in 4% (1/25) of cases clonality was showed in a polyclonal pattern. In B-HLs, 82% (9/11) of cases showed clonality and 18% (2/11) of the cases showed polyclonality. (The rate of positive clonality observed was 64.3% (9/14) of cases with B-LPD and in 35.7% (5/14) of cases clonality was not detected in any of immunoglobulin gene family (FR1 , FR2 ,FR3). In groups with DLBCL, clonality was detected in 95% (19/20) of the cases) This makes no sense. In patients diagnosed with FL and MALTs, 100% of cases showed clonality for complete IGH. Our study revealed that Euro Clonality BIOMED-2 protocols could be considered as a valuable and reliable method for clonality detection, especially in IGH analysis.

J12.13
upregulation of stanniocalcin1 gene expression in colorectal cancer

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Introduction: Identification of the genes involved in the carcinogenesis of the colorectal cancer could be useful for identifying of diagnosing biomarkers and improvement of treatment. Stanniocalcin1 is a glycoprotein hormone that is known to be involved in various biological function especially calcium hemostasis.Its upregulation has been shown with various cancers in several studies however its main role is not clear in cancer progression. In this study we assessed the expression of STC1 gene in colorectal cancer for the first time in Iran.
Material and methods: Tumor tissues and adjacent tissues were obtained from 48 colorectal carcinoma patients who undergone partial or total colectomy for CRC. The relative expression of STC1 was measured by using quantitative RT-PCR. GAPDH was used as a housekeeping gene. The Relative Expression Statistical Tool (REST) and SPSS software were applied for data analysis.
Results: Relative mRNA levels of STC1 were significantly higher in tumor tissues in comparison with margins (p value =0.025).
Conclusion: Our results showed that STC1 gene expression significantly increases in tumor tissues and therefore, it may be helpful as a molecular biomarker for early diagnosis of CRC.

J12.14
The EGFR mutational status screening in Romanian lung adenocarcinoma patients

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The personalized medicine (tyrosine-kinase inhibitors-TKI) represents an alternative to the advanced lung cancer patient's healthcare. Nowadays, the screening of the EGFR gene (which codifies a protein-kinase), has become a TKI-therapy selection tool. In non-small-cell lung carcinomas, the TKI-therapy response depends, additionally, on hystopatological subtype, the most suitable being adenocarcinoma (ADK).
The study aim was to analysis the EGFR mutational status in ADK patients which makes them susceptible for EGFR TKI-therapy.
DNA was extracted from formalin-fixed paraffin embedded tumor tissues (85 biopsies and 222 resections) from 307 Caucasians patients in different ADK stage (185M:122F, enrolled from December 2013-February 2015). EGFR mutations screening (exons 18-21) was performed by PCR reverse-hybridization (n=144) and ARMS-PCR (n=163).
EGFR mutations were detected in 50 (16,29%) patients (35 with primary and 15 with secondary tumors, mostly TTF-1 positive, tumor cell between 90% - 0,5%, sex distribution of 1M:1.39F, patients age 35-87 year old, average age at screening time-61,17±10,15). The most frequent mutations, which confer sensitivity to TKI therapy, were identified in exons 19 (62%) and 21 (24%). Less frequent were detected mutations in exon 18 (G719S) (6%), and the compound mutation p.E746_A750del/p.L858R (2%). The resistance mutation T790M (exon 20) was detected in 3 cases, in singlet (2%) or doublet condition (4%). The mutation T790M/EGFR-sensitive denotes acquired resistance post TKI-therapy (erlotinib), both high-stage ADK women having a progressive disease evolution.
Our results, in accordance with international data, showed that the EGFR mutational status detected by PCR-based method screening is a sensitive and helpful tool for the clinical decision in the ADK anti-EGFR personalized therapy.

J12.15
Discordance of HER2 status in primary breast carcinomas and distant metastatic sites

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Background: Assessment of human epidermal growth factor receptor 2 (HER2) status in patients with breast carcinoma (BC) is commonly performed using neoplastic tissue from the primary tumor. There are few data regarding the HER-2 status in the corresponding distant metastases. Several papers have shown that HER2 status may be different in metastatic lesions when compared with the primary tumor, and this discrepancy is more frequently found in distant metastases than in locoregional ones.
Methods: HER-2 status in 47 patients with a primary breast tumour (at different time of therapy stages) and at least one distant metastatic (bone, brain, lung, liver, or other) lesion was analysed by immunohistochemistry (IHC) and chromogenic in situ hybridization (CISH).
Results: The overall concordance rate for HER2 was 91.48%. Thirty-four cases were concordantly HER2-negative in primary BC and distal metastases, nine cases were HER2-positive in both primary and metastatic tumors, and 4 cases were discordantly HER2-positive in the primary BC and HER2-negative in the metastases.
Conclusion: Simultaneous determination of HER2 in BC and corresponding distant metastases is not mandatory, but may influence the therapeutic management. Discordance in HER2 status may be found not only between primary BC and its metastases, but also between consecutive relapses of the same tumor.
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J12.16
Hypermethylation of KISS1 and EDNRB promoters as predictors of disease progression in patients affected with sporadic colorectal cancer

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Background & objective: The incidence of Colorectal Cancer (CRC) is increasing rapidly world-wide, especially among the ageing. This sentence makes no sense (The accumulation of genetic and epigenetic changes are primarily occur in CRC, here on, the methylation profiles are altering in promoter KISS1 and EDNRB genes). In many cancers, KISS1 and EDNRB act as metastasis suppressor and tumor suppressor genes, respectively. This study aimed to examine the promoter hypermethylation status of KISS1 and EDNRB genes for predicting CRC progression.
Materials & methods: Total DNA was extracted from 45 paired fresh CRC samples and adjacent normal tissue, and then treated by bisulfite. Finally, DNA methylation status of KISS1 and EDNRB genes were determined by using the Methylation-Sensitive High-Resolution Melting method (MS-HRM).
Results: Primary data in this study showed that there are statistically significant differences in the methylation status of KISS1 gene between CRC samples and adjacent normal tissue (p-value=0.03). However, we observed no significant association in promoter methylation of the EDNRB gene (p-value=0.1).
Conclusion: KISS1 promoter hypermethylation might be a candidate biomarker for predicting CRC progression. Furthermore, to determine the use of EDNRB promoter hypermethylation as a predictor, other studies with more samples are necessary.

J12.17
Molecular analysis of the expression of miR-221 in tumor and marginal tissues of breast cancer patients

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Identification of biomarkers is important not only for cancer diagnosis, prognosis and treatment, but also in providing new insights into cancer biology. The aim of this study was to evaluate the clinical significance of miR-221 in patients with breast cancer. The miR-221 has been demonstrated to function as an oncomiR in human cancers. miR-221 promotes epithelial-to-mesenchymal transition (EMT) and confers tamoxifen resistance in breast cancer. However, the effects and mechanisms by which miR-221 regulates breast cancer aggressiveness remain unclear. The real-time reverse transcription-polymerase chain reaction was used to examine miR-221 levels prospectively in 40 pairs of samples of breast tumor tissue and adjacent noncancerous tissue. In addition, the relationship between miR-221 levels and clinicopathological features was explored. The capability of miR-221 to function as a tumor marker was also examined. miR-221 expression levels showed significant changes overall. However, miR-221 was significantly upregulated in a group of breast tumor tissue samples compared with matched noncancerous tissue samples.
We consider that miR-221 act as promising biomarkers for breast cancer and it would offer a new way in molecular targeting cancer treatment. Therefore, miR-221 may contribute to the aggressive clinical behavior of basal-like breast cancers.

J12.18
Molecular analysis of the expression of microRNA-222 in tumor and marginal tissues of breast cancer patients

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Breast cancer is a common malignancy and a leading cause of mortality due to cancer among woman. About 1.3 milion females develop breast cancer each year. microRNAs are small noncoding RNAs that regulate genes at post transcriptional level. Misexpression of miRNAs have been associated with tumorogenesis in various cancers including breast cancer . Emerging evidence has shown associations of microRNA-222 (miR-222) with crucial cell processes such as the epithelial-mesenchymal transition (EMT) and aberrant expression with tumorogenesis in many types of human malignancy. the aim of this study was to evaluate the clinical significance of miR-222 in patient with breast cancer. This study characterized the contribution of miR-222 to the breast cancer(BC) tumorogenesis. We evaluate the expression level of miR-222 in marginal and tumor tissue of 40 breast cancer patients using real time- PCR. miR-222 was significantly upregulated in tumor tissues compared with noncancerous tissue samples. Therefore, targeting miR-222 and its activators might be a promising therapeutic option to prevent malignant progression toward metastasis.

J12.19
Complex translocation involving Philadelphia chromosome in a patient with chronic myeloid leukemia

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The Philadelphia (Ph) chromosome is the cytogenetic marker in chronic myeloid leukemia (CML). However, about 5-10% of Ph positive patients with CML show variant translocations. We describe a patient with CML with a rare translocation between chromosomes 9, 10, 18 and 22. The patient is a 64 years old male diagnosed in 2004 with CML in blast phase. The patient had multiple hospitalizations and showed resistance to various therapies. In January 2014 we performed cytogenetic and molecular analysis in our laboratory. Molecular testing identified a molecular response of 62.69% of the BCR-ABL fusion transcript. Because of the patient's therapy resistance we performed molecular testing for the detection of mutations in the BCR-ABL1 transcript in the region corresponding to the BCR-ABL1 TK protein. The c.951C>A mutation was detected corresponding with the p.F317L phenotype associated with resistance to treatment with certain tyrosine kinase inhibitors. Conventional cytogenetic analysis at a 350 bands resolution initially revealed a 46,XY,t(9;10;22)(q34;q22;q11) karyotype in all the analyzed metaphases. To better characterize this translocation we performed a metaphase FISH analysis with BCR-ABL1 probes (MetaSystems). This revealed the involvement of the short arms of chromosome 18 in the complex translocation, as confirmed later by adding the centromeric probe for chromosome 18.
The formation mechanisms of these translocations, and their clinical significance is still unclear, and resistance to therapy of patients with these complex translocations remains to be studied.

J12.20
Polymorphism of p53 Gene Codon 72 in Endometrial Cancer: Correlation with Tumor Grade and Histological Type

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Background: Endometrial cancer is the fourth most common cancer among women in developed countries. Patients with endometrial cancer may benefit from systemic chemotherapy alone or in combination with targeted therapies if the disease is clinically diagnosed prior to spread and metastasis to other organs. The aim of this study was to evaluate the prognostic role of p53 polymorphism and its correlation with tumor grade in human uterine endometrial carcinomas. Materials and Methods: A total of 75 patients with endometrial carcinomas were studied for possible mutations in exon 4 of the p53 gene using polymerase chain reaction and restricting fragment length polymorphism techniques and sequencing. Results: In recent study, The rate of homozygote genotype of pro/pro or Arg/Arg in high grade group was higher than in comparison with low grade one. In addition samples that were undigested in RFLP, showed mutation in exone 4. Conclusions: Our findings showed that high grade endometrial carcinomas are highly associated with TP53 polymorphisms in comparison with low grades.

J12.21
Isochromosome14q ıs a non-random cytogenetic abnormalıty ın MDS

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Myelodysplastic syndromes(MDS) are heterogenous group of clonal disorders characterized by dysplasia in one or more myeloid cell lineages, inefficient hematopoiesis, peripheral cytopenies and an increased risk of transformation to AML. MDS frequently occurs in adults, particularly the elderly. The annual incidence of MDS is about four cases per 100.000 people. Cytopenia(s), bone marrow morphology and cytogenetics are central to the diagnosis of myelodysplastic syndrome.
The presence of isolated gain of a chromosome is reported in 6-7% of human malignancies; the most common of which in myeloid neoplasms are chromosomes 8, 9, 13, and 21. Trisomy 14 (trisomy 14 or isochromosome 14) as the sole cytogenetic abnormality is rather uncommon and has been reported in myeloid disorders such as MDS, myeloproliferative disorders, atypical chronic myeloid leukemia and AML.
A 60-year-old female who had a history of chronic kidney disease with

amyloidosis had been hospitalizated for haemodialysis ten months ago. She was consulted to haematology for anemia and reduced white blood cells and neutrophil. The bone marrow biopsy was hypercellular and revealed eritroid dysplasia. Her bone marrow cytogenetic analyses was normal. The patient was diagnosed as MDS. Seven months later rebiopsy was performed. Bone marrow karyotype was as 46,XX,i(14)(q10) [4]/46,XX[8] and AML-MDS FISH panel was normal. Patient is stil being followed.
The clinicopathologic futures and prognostic importance of trisomy 14 has not been well described so that we report this case in order to contribute the literature.

J12.22

Multidisciplinary Consultative Meetings for breast monitoring of femals who have a high risk of developping breast cancer in France : evaluation of strenghts and weaknesses, proposition of improvement

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The French National Cancer Institute published in its annual report of 2013 (Summary of 2013 oncogenetic activity - Consultations and laboratories, dec 2014) that there are more than 40.000 patients in France with a mutation implicate in hereditary predisposition of cancer, including more than 17.000 concerning BRCA gene.
Since its renewal mission in 2012, the French National Cancer Institute supports 17 projects to promote breast and ovarian monitoring for femals who have a high risk of developping breast cancer .
Four main missions are to be conducted: 1) develop an individualized monitoring of people hereditarily predisposed to cancer; 2) coordinate their breast and ovarian monitoring in regional or interregional level; 3) ensure and facilitate access to multidisciplinary skills, either within the institution (or institutions) holder of the project, or outside; 4) ensure resort activity and expertise for difficult cases.
We initiated a questionnaire to assess in France the faisability to the fourth mission. We sent a link to an online survey to french oncogeneticists thanks to a mailing list. The first part of the survey evaluates the expanded and modalities of implementation of the Multidisciplinary Consultative Meetings. The second part evaluates the interest of the development of these Multidisciplinary Consultative Meetings.
The aim of this article is to identify strenghts and weaknesses of these Multidisciplinary Consultative Meetings and to propose improvement.

J12.23

Study the genetic and epigenetic biomarkers in Iranian patients with colorectal cancer (CRC) in comparison with normal individuals

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Colorectal cancer (CRC) is the third most common cancer worldwide. CRC develops with progressive accumulation of genetic mutations. Stool DNA test as a non-invasive test, has high sensitivity and specificity for diagnosis of cancerous and pre-cancerous lesions and is associated with more acceptability of patient than invasive tests. Tumor shedding cells contain genetic markers which have significant role in CRC diagnosis. No single molecular marker has been recognized and validated for detection of all types of colorectal cancers. The aim of this survey is to study the genetic and epigenetic markers in colorectal cancer patients and comparison with control individuals to reach a multi-markers panel with reasonable sensitivity and specificity.
Colonoscopy is performed on patients with sporadic CRC and control individuals, after obtaining consent form and questionnaires. From all patients, stool, tumoral and normal tissue (or blood) and from all control, stool and blood are taken. Following DNA extraction from stool, tissue and blood, the samples are subjected for *Kras*, *BAT-26*, long DNA and *Vimentin* markers detection. Finally all markers (individually or in combination) are used to calculate the sensitivity and specificity of the test.
So far samples from 13 CRC and 48 control individuals have been collected. DNA from all tissue and blood samples were extracted and quantified. Long DNA detection was optimized in stool DNA. *BAT-26* detection is optimizing in blood and tissue DNA. Sample collection and optimization of other markers is underway.
Keywords: Colorectal cancer, molecular marker, Iran

J12.24

CAT, *GPX1*, and *GSTP1* genetic polymorphisms and the risk of acute myeloid leukemia in Romanian patients

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Reactive oxygen species (ROS) are associated with oxidative damage at the DNA level and thus may lead to genomic instability and to an increased the risk of developing malignant hemopathias.
Catalase (CAT)and glutathione peroxidase (GPX) are implicated in the defence mechanisms against oxidative stress.
Glutathione S-transferases (GST) enzymes, are responsible are by detoxification for the xenobotics' and thus protecting against oxidative damage produced by high levels of ROS.
The purpose of our study was the investigation the relationships between namely CAT C262T, GPX1 Pro198Leu as well as GSTP1 Ile105Val polymorphisms and the risk of acute myeloid leukemia (AML).
Genotyping of the mentioned polymorphisms were based on a multiplex polymerase-chain-reactions and restriction fragment length polymorphism (PCR-RFLP) methods in 100 AML patients and 200 healthy controls. We observed that variant genotypes of CAT C262T gene polymorphism were not associated with the risk of AML. Our findings showed that the variant homozygous genotypes of GPX1 Pro198Leu and GSTP1 Ile105Val polymorphisms were associated with an increased risk of developing AML ($p<0.0001$, $p=0.012$). The combined variant genotypes of all three investigated polymorphisms were analyzed in relation to FLT3 and
DNMT3A gene mutations. No significant associations between investigated polymorphisms and these parameters (p-value > 0.05)were found.
In conclusion, our data suggests that GPX1 Pro198Leu and GSTP1 Ile105Val variants seem to have an important role in development of AML.
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J12.25

der(19)t(1;19) in Childhood Acute Lymphoblastic Leukemia Patient

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t(1;19)(q23;p13) comprises approximately 5% of the translocations observed in childhood and adult acute lymphoblastic leukaemias (ALLs). It occurs either balanced t(1;19) or unbalanced der(19)t(1;19) where the TCF3/PBX1 fusion protein is associated with pre-B cell immunophenotype in both situations. Here we present 4 years old girl who was referred because of weakness and bone pain. She had hepatosplenomegaly and ecchymosis lesions at lower limbs. After routine laboratory investigations and flow cytometry she was diagnosed as pre B ALL. Fluorescence in situ hybridization (FISH) analysis at diagnosis revealed mosaic pattern which included 35% of balanced t(1;19) and 16% of unbalanced der(19)t(1;19). Following ALLIC BFM 2002 treatment protocol control FISH analysis was revealed chromosomal constitution normal. However 21% of der(19)t(1;19) was detected before reinduction therapy. She is still receiving maintenance treatment which is planned to continue for 6 months. Literature search revealed that derivative chromosome 19 is more common than balanced t(1;19). In conclusion here we present a further pre B ALL patient which is characterised by an interesting shifted translocation pattern with good prognosis.

J12.26

EMT gene expression signature in colorectal cancer with liver metastatic or peritoneal carcinomatosis

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Aim
Colorectal cancer (CRC) has been considered a molecularly heterogeneous disease. Investigation of gene expression signature in CRC is an effective approach for prognosis of disease. The analysis of EMT (epithelial- mesenchymal transition) program classifies colon tumors on two molecular subtypes: epithelial and mesenchymal.
Methods

We investigated 38 patients with colon or rectal cancers T3-4N0-2cM1. The gene expression signature (5 genes) was studied to 8 tumor samples and its liver metastatic (MTS), as well 20 tumor samples and its peritoneal carcinomatosis nodules (PC) by RT-PCR.
Results
Samples were characterized as mesenchymal subtype if detected: high level of ZEB1, ZEB2, VIM, SFRP2 and low level of CDH1. Tumor of mesenchymal subtypes were found in: 1 of 18 and 8 of 20 samples with liver MTS and PC, respectively. However, its MTS node was not mesenchymal phenotypes, whereas all of eight PC nodules had been mesenchymal subtypes. Concordance epithelial phenotype between tumors and nodules were observed for 12 and 3 samples with MTS and PC, respectively. Finally, five of 18 MTS and 9 of 20 PC nodules were corresponded to mesenchymal subtypes.
Conclusion
Our data confirm the notion that the occurrence and development of liver MTS and PC are different by the pathogenetic mechanism.

J12.27
Study of the correlation between p53 Arg72Pro and MDM4rs4245739 - Polymorphisms and Breast Cancer among Iranian-Azeri population

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Abstract
Background: Breast cancer is a clinically heterogeneous and complex disorder and its transformation can occur in different shapes which may be manifested as identical clinicopathologic indices. MDM4 is a negative regulator of the p53 tumor suppression pathway. Different researchers have revealed that the rs4245739A>C polymorphism of MDM4 in 3-untranslated regions makes it a miR-191 target site which leads to lower MDM4 expression. On the other side, the importance of Arg72Pro polymorphism of p53 in breast cancer has been proven. The aim of this study was to therefore realize the correlation of these SNPs and the risk of breast cancer in East-Azerbaijan, Iran.
Methods: 199 healthy controls and 206 women with breast cancer from Eastern-Azerbaijan, Iran were included. Tetra-ARMS PCR was employed in order to detect alleles of both positions. SPSS for Windows (version 22.0, IBM SPSS Inc., USA) and the SHEsis online software was exerted for allele typing, genotyping and haplotype analysis.
Results: Different alleles of both MDM4 rs4245739 and p53 Arg72Pro had no significant frequency in cases when compared to the control (P>0.05). Also, genotypes of neither MDM4 rs4245739 nor p53 Arg72Pro were shown to increase or decrease breast cancer risk in patients in comparison to healthy women. Gene-gene interaction also could not significantly affect the risk of breast cancer.
Conclusion: Our ungoing study revealed that two genetic variants, MDM4 rs4245739 and p53 Arg72Pro polymorphisms failed to be associated, alone and in their combination, with the risk of breast cancer development in Iranian-Azeri Patients. However, additional well-designed studies on larger populations are required to validate this association

J12.28
Verification of influence of C1236T (rs1128503) polymorphism in the ABCB1 gene in chemotherapeutic response and clinical variability in the breast cancer

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Introduction: Different polymorphisms in genes involved in the metabolism of drugs, has been evaluated and associated in the response to chemotherapy. Among the genes, *ABCB1*, with important function of efflux drug, has been studied. In the gene, the C1236T polymorphism (rs1128503) has been evaluated and presented intriguing results, including in the breast cancer. The objective of the present study was to associate the response to chemotherapy in patients with breast cancer and the clinical variability of the disease with the C1236T polymorphism in the *ABCB1*gene
Material and methods: The study enrolled 100 female patients with breast cancer. The clinical markers included were: race, use of oral contraceptive, breastfeeding, hormone replacement therapy, smoking, alcoholism, hypertension, diabetes mellitus, histological type, histological and nuclear grades, tumor classification by TNM, clinical stage, radiation therapy, hormone therapy, patient status, age, menarche, menopause, age at first live birth, height, weight, estrogen and progesterone receptors, and chemotherapy. **Results and discussion:** For genotypic analysis, the polymorphism was in Hardy-Weinberg equilibrium (p>0.05). In the present study, we found no association of the polymorphism analyzed with the clinical presentation of patients with breast cancer, except for race (Caucasian versus non-Caucasian) patients, however there was no positive oddsratio after the data correction. However, there was no association with response to chemotherapy used in patients followed in our center. In conclusion, in this study, the C1236T polymorphism was associated with patient's race, time to onset of menopause and the percentage of progesterone receptors in patients with breast cancer.

J12.29
Prognostic and predictive significance of the bcl -2/IgH translocation in malignant follicular lymphomas

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In Europe, follicular lymphomas constitute up to 30% of non-Hodgkin lymphomas.
Prognostic markers, identified by 2 different working groups, immunohistochemical (CD10, bcl 2 positivity) and molecular the bcl 2/IgH hybrid gene, the t(14;18)(q32:q21.
The aim of our study was to analized the cytogenetical aberrations in malignant follicular lymphomas, in order to identify the prognostic and predictive value of bcl2-2/IgH translocation in these malignancies.
Material and method:
We conducted a study on 79 patients with follicular lymphomas. The study was carried out on tissue samples selected from the "Victor Babes" National Institute of Pathology files. These samples were formalin-fixed paraffin embedded-tissues, routinely processed for histology.
The t(14;18) translocation, realized by the bcl 2/IgH rearrangement, supposedly occurs in almost all follicular lymphomas (FL) can be detected by FISH methods
We employed the PathVysion LSI IGH Spectrum Green/ LSI bcl2 Spectrum Orange (VYSIS) kit.
Results:
Asingnificant positive correlation was found between the IHC positivity for bcl 2 and the FISH detection of t (14;18) translocation (p=0,04).
Twenty two cases were selected for FISH analysis, five cases were excluded because of the processing artifacts , ten of the 17 cases without artifacts (58.8 %) presented t(14:18) translocation: two cases of FL grade 1-2, two cases of FL grade 1, six cases of FL grade 3.
In 66.6% of cases with t(14;18) translocation, the immunohistochemical reaction for bcl 2 protein was positive.
Bcl2 t(14;18) translocation plays an important role in the pathogenesis of follicular lymphoma, and is an important tool in the diagnosis and treatment.

J12.30
NTRK3 and SEPT9 promoter hypermethylation in patients with sporadic colorectal cancer can act as early diagnostic biomarkers

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Background & objective: Colorectal cancer (CRC) is one of the most common malignancies and the third leading cause of cancer mortality worldwide. Timely detection of CRC in patients in the earlier stages of disease provides the highest rate of survival. Epigenetic alterations which play a significant role in tumorogenesis are prevalent among CRC patients and represent as the primary modifications of cancer cells, therefore, these alterations are thought to hold great promise as tumor biomarkers. It has been shown that NTRK3 and SEPT9 gene promoters are hypermethylated and can be used as biomarkers for early detection of CRC. In this study we analyzed promoter methylation status of these genes' in CRC patients.
Materials & methods: Genomic DNA was extracted from 45 CRC and paired adjacent normal tissues and undergone bisulfite conversion. Finally, the methylation status of NTRK3 and SEPT9 were defined with the Methylation-Specific High-Resolution Melting (MS-HRM) method.
Results: Our results showed that there are statistically significant differences in the methylation status of NTRK3 and especially SEPT9 between CRC and adjacent normal tissues (p-value=0.05 and 0.02 respectively).
Conclusion: SEPT9 promoter hypermethylation might play a role as a trusted biomarker for detection of CRC development; however, to validate the biomarker potential of NTRK3 there is a substantial need for further investigations.

J12.31

VEGF gene expression level in tumor and non tumor colorectal tissues

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Colorectal cancer (CRC) is one of the most lethal malignancies in the world. It is the third most common cancer and a leading cause of cancer-related death. There has been much interest in the development and use of molecular-based research aimed at identifying biomarkers for the diagnosis of the disease. Vascular endothelial growth factor (VEGF) has a crucial role in tumor angiogenesis and is found to be overexpressed and involved in the development and progression of CRC. Moreover, its over-expression is frequently considered as a marker of both, a poor prognosis and of an aggressive tumor phenotype. Thus, the aims of this study were to quantitatively examine the expression of VEGF in tumor and marginal tissue in CRC and to correlate their expression levels with clinico-pathological variables. Human colorectal cancer tissues (n=45) and non-tumor (marginal) tissues (n=45) which were all pathologically diagnosed, were obtained after surgery. After RNA extracting from frozen pieces for gene amplification, the expression of VEGF were assessed using Real-Time PCR subsequently after cDNA synthesis by polyA RT-PCR. VEGF expression was significantly raised in the tumor than in the non-tumor tissues ($P < 0.005$). And its expression was lower in patients with stage I than in patients with higher clinical stages. In conclusion, our data suggests that dysregulated VEGF gene expression is potentially involved in the carcinogenesis of CRC and the clinical outcome could be related to its over-expression in CRC patients. Hence, VEGF may has prognostic and therapeutic values in them.

J12.32

Differential effects of radiotherapy on normal cell, cancer stem cells and cancer cells

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Radiotherapy is an important method for treatment of cancer. In radiation oncology preclinical models such as cell culture methods are necessary for cancer research. For this purpose, human head and neck stem cell line (HNSC), head and neck cell line (FADU), and as control group, epithelial cell line (WSS-1) have been studied. Dose ranges determined by using clonogenic assay, cell proliferation assay, DNA double break analysis, apoptosis analysis and cell cycle analysis. All cell lines were exposed to 0,2,4,6,8 and 10Gy and cell amount were variable for each. When evaluated the effectiveness of radiotherapy on HNSC and FADU, they were similar according to the clonogenic assay results, there was a significant relationship between dose and survival and 10% survival was close to 8Gy and there was a significant relationship between dose and survival (p= 2,16E-4) and 10% survival was close to 4 Gy on WSS-1. According to the apoptotic activity, for HNSC, apoptosis at 48 hours was induced compared to control between 2-10Gy, for FADU, at 72 hours, 4-10Gy and for WSS-1, between 24-72 hours at 8 and 10Gy. A significant increase was detected in the formation of γh2ax foci after DNA double strand breaks in HNSC and FADU at 48 hours, between 2-8 Gy, compared to control and there was no DNA double strand breaks in WSS-1. On the efect of cell cycle, in HNSC and FADU, arrested at G2/M between 2-10Gy and in WSS-1, arrested at S and G2/M between 24-72. hours. Radiotherapy shows differential effects on normal tissuse compare with cancer stem cells and cancer cells.TUBİTAK

J12.33

KRAS, BRAF oncogene mutations and tissue spesific promoter hypermethylation of tumor supressor HIC-1, P16, DAPK1, SFRP2 and MGMT genes in colorectal cancer patients

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Aims: Within the context of this study, frequencies of oncogene KRAS and BRAF mutations, promoter hypermethylation profiles of tumor suppressor genes HIC1, SFRP2, P16, DAPK1 and MGMT and possible associations between hypermethylations of these genes and KRAS and BRAF mutations in colorectal patients were aimed to find out.

Methods: 93 colorectal cancer tissue and 14 normal colon mucosa were included in the study. Common twelve KRAS gene mutation were investigated with using reverse-hybridisation strip assay method. BRAF V600E mutations were investigated with using RFLP method.

Results: Hypermethylation status of tumor suppressor genes (SFRP2, DAPK1, MGMT, HIC1 and p16) were detected by using reverse-hybridisation strip assay method after bisulphite modification of DNA. In the patient group, KRAS and BRAF mutation frequency were determined as 54.84% (n=51) and 12.9% (n=12) respectively. Promoter hypermethylation frequencies of tumor suppressor genes SFRP2, DAPK1, MGMT, HIC1 and p16 were determined as 66.7%, 45.2%, 40.9%, 40.9%, 15.1% respectively. No statistically significant associations were found between KRAS gene mutation and tumor suppressor gene hypermethylation. Statistically significant associations were found between BRAF gene mutations and SFRP2 and p16 tumor suppressor gene hypermethylation ($p=0.005$ for SFRP2 and $p=0.016$ for p16). In terms of the tumor location, SFRP2 ($p = 0.017$) and MGMT ($p = 0.013$) genes have statistically significantly higher promoter hypermethylation in colon compared to the rectum.

Conclusions: KRAS mutations and SFRP2 tumor suppressor gene promoter hypermethylations play important role in colorectal cancer oncogenesis. BRAF V600E mutation was found to be associated with tumor suppressor gene hypermethylation.

J12.34

Increase of the risk of differentiated thyroid carcinoma in the Great Poland population by c.470T>C CHEK2 missense variant

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Differentiated thyroid carcinoma (DTC)originates from thyroid follicular epithelial cells and belongs to a group of slowly progressing tumors with a relatively good prognosis.However, recurrences and metastases are a serious problem in advanced stages.

The majority of DTC are sporadic but a few alleles increasing the cancer risk are known. One of them is the c.470T>C (p.I157T, rs17879961) missense substitution in the CHEK2 gene.

The aim of this study was to investigate whether this specific CHEK2 alteration, c.470T>C, predisposes the Great Poland (Wielkopolska) population to thyroid cancer.

602 differentiated thyroid carcinoma patients and 829 controls randomly selected from population were genotyped for the presence of the c.470C allele using pyrosequencing. Hardy-Weinberg Equilibrium (HWE) was tested for both groups by chi-square distribution and Fisher's exact test. The odds ratios (ORs), 95% confidence intervals (CIs), and p-values were calculated using the R software.

The results of genotyping showed the presence of the c.470C allele in 51 patients with a frequency of 4.49%, while in a controls in 42 with a frequency of 2.53%. We demonstrated that in the Great Poland population the c.470C CHEK2 variant increases the risk of developing differentiated thyroid cancer almost twice (OR=1.81, p=0.004). The risk of papillary thyroid carcinoma in female patients homozygous for the c.470C allele was shown to increase almost 13-fold (OR=12.81,p=0.019).<br Identification of c.470C CHEK2 gene variant ought to be taken into account by healthcare policymakers. Future well-designed and larger population studies are of great value in confirming these findings. Moreover, a combination of genetic factors together with environmental exposures should also be considered.

J12.35

Widespread Mutation Detection of RET Proto-oncogene in Medullary Thyroid Carcinoma

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Introduction: Thyroid cancer is the most common endocrine malignancy. Medullary Thyroid Carcinoma (MTC) is one of the most aggressive thyroid tumors which occur in both hereditary (25%) and sporadic (75%) forms. Mutations of the RET proto-oncogene in MTC development have been well

demonstrated. The aim of the study was to investigate the mutational spectrum of exons 2, 3, 5, 8, and 10-19 of RET Proto-oncogene in MTC patients. Material and methods: this retrospective study has been started since 2001 in Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, and Tehran, Iran. 399 participants, including 233 patients (176sMTC, 40FMTC, 8MEN2A, 4MEN2B, 5pheochromocitoma), and 166 relatives were evaluated. Genomic DNA was extracted by the standard Salting Out/ProteinaseK method and Mutation detection was performed through direct DNA sequencing. Results: Totally, 102 mutations were identified in RET exons 10, 11, 13-16 and 18. Furthermore, 357 Single Nucleotide Polymorphism (SNP) were found in exons 2, 3, 13, 14, 15. Interestingly, G691S SNP and S904S SNP were 100% in linkage disequilibrium in 131 patients and 87 relatives (Table 1). The most common mutation in our population were C634Y and C634R (4%) whereas C618R, C618S, C620G, L887L mutations had rare allele frequency (0.3%).Discussion: Exon 11 and after that exon 10 were the most frequently mutated exons of RET proto-oncogene in MTC patients in Iranian population. We did not find any substantial mutation in non-main exons of RET. Therefor these exons may not be recommended for RET genetic screening test in MTC patients and their relatives.

J12.36

Early detection of genome changes with I-FISH in patients with cellular atypia in urinary sediment

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Urinary bladder carcinoma is one of the leading cancers in male population. With highly sensitive, specific and non-invasive techniques of molecular biology such as interphase FISH (I-FISH) it is possible to detect genome changes in urine sediment cells and therefore distinguish between malignant and benign atypical cells. The aim of the study was to detect and confirm the presence of genome changes in atypical urine sediment cells. The I-FISH technique identified numeric and structural changes (CEP3, CEP7, CEP17 and LSI9p21) in urine samples with confirmed atypical cells. The UroVysion test (FDA approved, CE) allowed us to detect various changes in the genome of one atypical cell simultaneously. 270 urine samples in which atypical cells were present were analyzed. A positive urine finding was confirmed in 113 patients (42%). In 24 patients (21%) both numeric and structural changes were present, while in 83 patients (74%) only numeric changes, and in 6 patients (5%) only structural changes were found. Conclusion: With the I-FISH technique it is possible to detect and monitor known genome changes in cells of urinary bladder cancer and in this way distinguish between benign and malignant atypical cells.

J12.37

Polymorphisms and allelic imbalance of the TP53 gene and sensitivity of gastric cancer patients to 5-FU chemotherapy.

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TP53 is a tumor suppressor gene involved in multiple pathways including apoptosis, cellular transcriptional control, and cell cycle regulation. Single nucleotide polymorphism (SNP) at codon 72 of the TP53 gene has been associated with the risk of developing various neoplasms.
We examined Arg > Pro in codon 72 (rs1042522) and 16 bp insertion in intron 3 (rs17878362) in 80 gastric cancer patients after surgical treatment and chemotherapy with 5-fluorouracil. Determination of allelic imbalance of the locus 17p13 (TP53) in tumor samples from 80 gastric cancer patients was performed by microsatellite analysis.
We have found the Pro/Pro genotype to be associated with poor prognosis, development of local recurrence, carcinomatosis, appearance of distant metastases during the first 3 years after surgery and 5-FU treatment ($p = 0.031$). There is a statistically significant increase in disease-free survival in patients with Arg/Arg genotype, compared to the Pro/Pro ($p = 0.013$) and a statistically significant increase in disease-free survival with genotype Arg/Pro vs. Pro/Pro ($p = 0,015$). In the study of the 16 bp insertion (rs17878362) in the patients after surgical treatment and chemotherapy we found no significant differences.
We have shown that the frequency of allelic imbalance of the locus 17p13 (TP53) in patients with intestinal gastric cancer was significantly higher than in patients diffuse type ($p = 0.016$).
We suggest that TP53 polymorphism rs1042522 can serve as individual prognostic factors of adjuvant treatment effectiveness in patients with locally advanced gastric cancer.

J12.38

RAD51D Mutations in Breast and Ovarian Cancer Families from Northeast of Spain.

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The identification of new predisposing genes to hereditary breast and ovarian cancer is essential to enable decisions about the treatment and follow up of this syndrome. Homologous Repair (HR) genes deserve special attention for two reasons: mutations on them could confer increased risk to develop Hereditary Breast and Ovarian Cancer and justify the use of PARP inhibitors in order to improve treatment effectiveness. RAD51D is a key player in HR pathway. Several studies concluded that RAD51D is a moderate penetrance susceptibility gene for ovarian cancer.
Our aim is to determine the prevalence of germline mutations of RAD51D in a cohort of Spanish Breast and Ovarian Cancer (BOC) families without BRCA mutations.
The screening for germline variants RAD51D was performed in 94 BOC families by High Resolution Melting. Mutations identified were confirmed by direct DNA sequencing.
A new frameshift mutation, c.94delG, has been identified in ovarian cancer case. In addition, other changes previously described have been found: four missense variants (c.494G>A, c.629C>T, c.695G>A y c.698A>G) and one intronic.83-4T>C. Bioinformatics analysis of missense substitutions predicted c.629C>T to be deleterious.
Genetic testing of RAD51D should be considered in high risk BOC families as far as preclinical studies have shown that RAD51D-deficient cells respond to PARP inhibition. The compassionate use of PARP inhibitors in high grade serous ovarian cancer patients should also be considered.

J12.39

First study of Braf V600E mutation's status in thyroid carcinoma in Tunisian population

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Introduction: Braf V600E mutation was associated with high risk clinicopathologic characteristics in patients with papillary thyroid carcinoma (PTC) in many populations. This molecular alteration may be a potential prognostic factor in PTC. In our study, we wanted to determinate the status of the Braf V600E mutation in thyroid carcinoma in Tunisian population.

Matriels and methods: Twenty four patients with primary thyroid carcinoma (11 follicular, 13 papillary) underwent surgery at H.Bourghiba Hospital in sfax, between january and december 2010. Real Time Polymerase Chain Reaction was used to detect Braf V600E mutation gene, exon 15 on chromosome 7q34, from paraffin embedded thyroid tumor specimens.

Results: Braf V600E mutation was detected in 53% of the PTC (7/13). Any mutation was found in 8/11 follicular thyroid carcinoma and DNA was non informative in three cases. In one case the mutation was detected in both the primary tumor and the nodule tissues.

Conclusion: Braf V600E mutation may be a common genetic alteration in the Tunisian population despite the low number of patients. Another study with large series including clinicopathologic characteristics is necessary to determine the impact of this molecular alteration in the prognostic of PTC in our population.

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J12.40

Application of whole-exome analysis to find molecular-genetic predictors of safe cancellation of targeted therapy in patients with chronic myeloid leukemia

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High efficacy of targeted therapy with tyrosine kinase inhibitors (TKI) led to major molecular response in more than 50% of chronic myeloid leukemia (CML) patients. After discontinuation of the therapy only 41% of CML patients keep molecular remission in the following 12 months. In order to identify genes with predictive significance for relapse-free survival after stopping targeted therapy in CML patients we performed exome sequencing using PGM Ion Torrent in DNA samples from 6 patients with stable long deep molecular remission (BCR-ABL less than 0.01%IS for 12 months) before discontinuing therapy. The patients were divided into groups with relapse (group 1, BCR-ABL more than 0.1%IS, n = 3) and without relapse (group 2, n = 3) after 24 months since therapy stopping. 371 unique common genetic variants were identified in the group 1, 366 unique common genetic variants - in the group 2. These variants were functionally annotated using Variant Effect Predictor. SIFT and PolyPhen scores were used for filtering of the potentially most meaningful variants: 12 in patients in group 1 and 18 in group 2. Among these selected variants we describe 4 genes related to cancer. In group 1 found variant in SPINT1 gene mutations in which are often associated with cancer. In the group 2 we found variants in the MAP2K3 gene, involved in carcinogenesis, WDR5, associated with leukemia, and CASC5, which expression is increased in a variety of tumor cells. Found exome variants are potential markers of persistent remission in CML patients after cancellation of TKI therapy.

J12.41
The prevalence of FLT3 internal tandem duplication in acute leukemia: A Tunisian experience

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The fms-like tyrosine kinase 3 (FLT3) gene is a member of the class III receptor tyrosine kinase family which plays an important role in hematopoiesis. Targeting the FMS-like tyrosine kinase receptor-3 (FLT3) in acute leukemia is mainly important. Mutations in the fms-like tyrosine kinase 3 (FLT3) genes, primarily the FLT3-internal tandem duplication (FLT3-ITD) is well established prognostic marker especially in acute myeloid leukemia (AML).
The present study investigated the molecular screening and incidence of FLT3-ITD in acute leukemia patients from Tunisia.
Genomic DNA was extracted from EDTA-anticoagulant blood samples from a total of 35 patients suffering from acute leukemia. After DNA extraction, the polymerase chain reaction using specific primers was conducted to screen the FLT3-ITD.
In acute lymphoblastic leukemia (ALL), 9 cases are LAL-B and median age is 24 years. In acute myeloid leukemia (AML), there were younger patients; M4 subtype and a leukocytosis. Chromosome abnormalities were detected in 23% (2 patients with AML and 5 with ALL) and are correlated with worse prognosis (very high risk and relapse). At molecular lever, never FLT3-ITD was detected both in AML and ALL.
Our findings suggest that FLT3 ITD is uncommon in Tunisian acute leukemia and do not affect clinical outcome.

J12.42
Analysis of IDH mutation, EGFR/MDM2 amplification, and INA expression in clinical diffuse gliomas

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Introduction: The diagnosis of gliomas is mainly based on the 2007 WHO classification, which suffers from a lack of precision and reproducibility. Nowadays, the search for genetic alterations in gliomas shows a potential role in diagnosis, prognosis and response to treatment.
The purpose of this study is to evaluate the presence and the frequency of some genetic biomarkers in Moroccan diffuse glioma patients.
Materials and Methods: 99 tumor samples were used for this study. They were obtained from patients diagnosed with glioma at the University Hospital Hassan II of Fez between January 2010 and December 2013.
Immunohistochemistry was performed with the antibody directed against the internexin alpha (INA) which is used as a surrogate marker of 1p / 19q codeletion in oligodendroglial tumors.
We performed a RT-PCR to study *EGFR* and *MDM2* genes amplification in glioblastomas and sequencing techniques to detect mutations in *IDH* genes in diffuse gliomas.
Results: INA immunostaining was found in 80% of patients presenting an oligodendroglial tumor. We identified *IDH1* mutation in 43 cases while one *IDH2* mutation was detected in an anaplasic oligodendroglioma.
The detection of EGFR/*MDM2* amplification with RT-PCR techniques is still in process.
This study will focus on the presence and the frequency of these markers. These data should be compared with literature.
Conclusion: The new molecular technology has allowed better classification of gliomas based on the alteration of specific genes for each tumor subtype. These molecular markers are necessary for accurate and objective differentiation and consequently for a more targeted therapy.

J12.43
Role of polymorphic fibroblast growth factor receptor Gene and Breast Cancer Risk

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Genetic factors related to cancer have been extensively studied and several polymor- phisms have been associated to breast cancer. Breast cancer (BC) is one of the most common causes of death among women, and second in Iran. The objectives of this study were to determine the frequency of the fibroblast growth factor receptor (FGF- R) Gene polymorphism in patients with breast cancer. For the first time, we evaluated these polymorphisms and effects on the breast cancer risk association in an Iranian sporadic population-based case-control study of 126 breast cancer cases and 160 con- trols using a PCR-RFLP-based assay. Analyses of affected and controls show that homozygote genotype FGFR4 Gly/Gly has the highest frequency in patients and control groups (30.4 and 18.9%). Genotype FGFR4 Gly/Gly most risk factor were in our population: ArgGly /GlyGly, OR= 2.359, 95% CI= 0.208 - 4.621, p=0.001; Arg- Arg /ArgGly, OR=0.412, 95% CL=0.082 - 0.547, p=0.078 , ArgArg /GlyGly, OR = 0.076,95%CI=0.030-0.189,p=0.

J12.44
A Jordanian trainee's perspective on cancer genetics services

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5.5 billion of the world's 7.1 billion population live in developing countries. Most of these people do not have access to services that could provide genetic counselling and diagnostic tests for familial cancer risk. It is expected that, over time, services currently available in developed countries will become increasingly available elsewhere.
King Hussein Cancer Center (KHCC) in Jordan is one of the most specialised centres in the Middle East dedicated to the treatment of paediatric and adult cancer patients. However, there is no comprehensive cancer genetics counselling or testing service.
The KHCC vision and strategic plan includes improving access to education, training, and research in collaboration with international centres. Our aim is to provide and implement service provision for cancer patients. Genetic counselling service will help cancer patients to access for genetic testing in line with international standards.
I have clinical attachment in the Yorkshire Regional Genetics Service based in Leeds, under the supervision of Dr Julian Adlard and his team. The future level of demand in Jordan is likely to increase as more members of the public become aware. There are challenges to introducing wider germline genetic testing. However, we are working to develop a Next Generation Service for genetic testing 'in house' in the near future.

J12.45
AML1 gene amplification in two TEL/AML1 positive acute lymphoblastic leukaemia cases

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TEL/AML1 (ETV6/RUNX1) fusion resulting from the translocation t(12;21)(p13;q22) constitutes the most common chimeric fusion gene in initial childhood acute lymphoblastic leukemia (ALL) (19-27%) and has been associated with good prognosis. Three secondary aberrations in TEL/AML1 positive ALL have been suspected to negatively influence outcome: deletion of the second TEL allele, gain of the second AML1 allele and duplication of the derivative chromosome 21 (der(21)).
In newly diagnosed two patients with TEL/AML1 positive ALL, additional (a) TEL loss, (b)TEL gain, (c) AML1 amplification (copy number greater than four), (d) combined TEL loss and AML1 gain were detected by FISH analysis.
RUNX1 is a transcription factor and targets key regulators of the hemato-

poiesis process (M-CSF R, IL3, neutrophil elastase, MPO, granzyme B, TCRs, and B-Cell receptors. It is possible that the expression levels of RUNX1 could explain the clinical heterogeneity of t(12;21) ALL cases. Indeed, it has been suspected that a RUNX1 gene copy number of four is associated with good prognosis. By contrast, whereas the amplification of RUNX1 to a copy number greater than four, which has been estimated to be the case in 2% of all pediatric ALL , may be characteristic of a subtype of B-ALL associated with a poor prognosis. Our data and published results on TEL/AML1 positive ALL with AML1 amplification and impact on prognosis were evaluated.

J12.46
A new BRCA 2 frameshift mutation detected on premenoposal woman with recurrent non metastatic breast cancer

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BRCA1 and BRCA2 genes are the most known tumor supressor genes associated with breast and ovarian cancer. Approximately 10% of breast and ovarian cancer patients have BRCA mutations (1,2). Genetic screening for patients with early onset breast canser (premenoposal or < age 50) and over cancer at any age, is increasingly rising in our country. Here we report a 41 year-old premenoposal woman who have had bilateral total mastectomy for invasive ductal carcinoma of right breast for 7 years ago and left breast for 2 years ago. DNA sequence analyses for BRCA 1 and 2 genes were performed for the patient having not any familial history. The analysis revealed a frameshift mutation on the sixth exon of BRCA 2 gene (c.506 ins A mutation). This new frameshift mutation is not reported on literature or any databases. The patient had genetic counseling and was advised to have family screening. The clinical impact of this mutation will become clear with the increase of the patients with the same mutation.

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J12.47
Autophagic effect of theranekron in gastric cancer cells

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Introduction: Gastric cancer is a common type that originates at the mucosa epithelia of the stomach and expands rapidly to the lining of the stomach. Theranekron is an alcoholic extract of the venom from Tarantula cubensis spider. Theranekron is a homeopathic remedy that is used as a pharmaceutical compound serving in veterinary medicine with outstanding success for its antiphlogistic, demarcative, necrotizing, and wound healing effects. Autophagy is a tightly regulated cell death mechanism involving the degradation of a cell's own components through the lysosomal machinery and activating the signal pathways during the distribution of the homeostasis. The aim of the present study was to evaluate the effects of theranekron in respect of autophagy in metastatic AGS and non-metastatic MKN-45 human gastric cell lines and control HEK-293 cells.
Materials and Methods: After appropriate treatment time and IC50 was determined, effect of theranekron on cell death was investigated using different concentrations of the drug. The autophagic effect of the drug was determined through the LC3-GFP translocation assay, qPCR technique for target genes, and also using rapamycin as a positive control.
Results: Theranekron decreased the cell viability in respect to increasing concentrations. Additionally, a significantly increased GFP accumulation was detected in the autophagosomes of the cells treated with theranekron compared to non-treated cells ($p<0,05$), indicating the presence of autophagy. The findings were confirmed by using qPCR.
Conclusions: Theranekron result in cell death and stimulate the autophagy process, but it is not specific for cancer cells since it represented similar results in control cells.

J12.48
Our experience of unaffected BRCA1/2 testing in the Peninsula

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In June 2013 NICE updated their recommended guidelines for BRCA1/2 testing. These new guidelines recommend that genetic testing should be available to an individual with 'no personal history of breast or ovarian cancer if their combined BRCA1 and BRCA2 mutation carrier probability is 10% or more and an affected relative is unavailable for testing' (NICE 2013).
Following this change in recommendations our department created a guideline for offering testing to unaffected individuals. We currently offer unaffected testing to individuals (both men and women) with an affected first degree relative, where there are no living affected relatives available for testing. The Manchester scoring system is used to calculate carrier probability, with testing being offered in families with a score of 20 or above. Boadicea may also be used as tool to help calculate risk. In addition, at least one of the cancers must be confirmed, ideally the most significant. We agreed that these patients should be seen by a Genetic counsellor and a consultant before testing, with results being given face to face. All patients will also be discussed at our cancer meetings.
Using these criteria we would expect a 10% or greater pickup of pathogenic mutations. We present our findings from testing over the past 14 months. We also discuss some of the challenges we have faced so far.

J12.49
The impact of 3'-UTR variant binding site of KRAS gene with let-7 micro RNA on metastasis in head and neck cancer

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Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer in the world and accounts for 90% of malignant neoplasia of the upper respiratory system. MicroRNAs (miRNAs) are highly conserved, small noncoding RNA molecules aberrantly expressed in various pathologies including cancer, regulation of tumour and metastasis associated genes. KRAS is very important oncogene, which leads to the overexpression of ras protein, suppression of apoptosis, promoting the pathogenesis and development of tumours. Variant (rs61764370) of the let-7 miRNA complementary site of KRAS gene 3'-untranslated region (KRAS-LCS6) has been shown to disrupt the ability of miRNAs to target genes resulting in differential target mRNA and protein expression. In the literature, there is data about a correlation of the KRAS expression with miRNA let-7 expression level, and also KRAS mutation and metastasis in different cancers. Yet, it is unknown about the impact of variation of KRAS-LCS6 site on metastasis pathway in HNSCC. In this study, the role of variant complementary site LCS6 of the let-7 miRNA on metastasis was evaluated in head and neck cancer. Using proliferation, invasion, migration, cancer stem cell and quantitative real-time PCR assays, we compared the effect of the rs61764370 (KRAS G12V-LCS6 T>G) in HEp-2 and HEK-293 cell lines transfected with KRASG12V -LCS6(T) (wild type), KRASG12V-LCS6 (G) (mutant) plasmids and empty pLenti-CMV-GFP-2A-puro plasmid, and also un-transfected cells for each type.
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J12.50
IL-33 Dependent ADAMTS5 Expression In Glioblastoma Multiforme

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Glioblastoma multiforme (GBM) is still one of the most deadly and incurable form of cancer, indicating a need for understanding the biology of GBM. In the present study we assessed the relationship between IL-33 and ADAMTS5 in GBM tumorigenesis. For this purpose, ADAMTS5 protein was initially determined in samples from GBM and low-grade tumor tissues (LGTt) and in samples from healthy brain tissues by western blot. We found that ADAMTS5 protein was significantly increased in both GBM and LGTt compared to healthy brain tissues (5,56 fold). Then, we performed primary tumor cell cultures derived from both GBM and LGTt. On these primary tumor cells, GFAP staining was performed as a marker of glial cells and found that GBM primary cells (GPc) were %92 GFAP positive. To evaluate the relationship between IL-33 and ADAMTS5, both low-grade primary tumor (LGPt) cells and GPc were treated with 10 ng/ml and 30 ng /ml IL-33 for 48 hours. Exposure of these cells to IL-33 for 48h resulted in a dose dependent increase in ADAMTS5 protein. In detail, a 9,98 fold increase in ADAMTS5 protein was observed after 30ng /ml IL-33 treatment of GPc. Similar results were obtained in LGPt cells as a 4,68 fold increase in ADAMTS5 protein was obtained after 30 ng/ml IL-33 treatment. As a conclusion, IL-33 seems to increase the expression of ADAMTS5 protein in GBM. Targeting IL-33 and

ADAMTS5 may be considered as a treatment strategy to improve prognosis and survival in GBM.
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J12.51
SCREENING OF BRCA GENE MUTATIONS IN MALE BREAST CANCER PATIENTS.

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Male breast cancer is a disease that affects approximately one in 1,000 men. Several risk factors influence the occurrence of this disease: age of diagnosis, unhealthy lifestyle and different genetic factors, such as mutations in BRCA genes. Mutations in BRCA2 gene are more frequent (incidence between 4-40%) than in BRCA1 gene (incidence less than 10%).
Our aim was to analyze the presence of pathogenic mutations in BRCA genes in 34 men with breast cancer divided in two groups: 15 with family history of breast cancer and 19 without it, all from the western part of the Spanish Castilla y León Community.
The analysis of point mutations was performed by PCR, CSGE and Sanger sequencing in both BRCA1 and BRCA2 genes. When no cause-disease mutations were detected, a further analysis of genomic rearrangements was performed using a MLPA test. All mutations detected in our study were found in BRCA2 gene and in males with family history. We found a nonsense mutation (c.145G>T) in one patient and the same frameshift mutation (c.2808_2811delACAA) in three others. Besides, we found 18 polymorphisms in BRCA2 and 10 in BRCA1 in patients of both groups. We did not find pathogenic mutations in patients without a family history.
In conclusion, we show that mutations tend to appear in the BRCA2 gene in a minority of patients with family history, which may be indicative of the existence of other genes associated with male breast cancer still unknown and should help to modify inclusion criteria in screening programs.
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J12.52
Splicing effect analysis of twenty BRCA1 and BRCA2 unclassified variants identified in breast/ovarian cancer patients from south Catalonia

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Introduction: Hereditary breast and ovarian cancer syndrome (HBOC) is mainly caused by pathogenic mutations in the *BRCA1* and *BRCA2* genes. The detection of unclassified variants (UVs) is one of the main difficulties in the diagnosis of this syndrome. The pathological characterization of these unclassified variants is an indispensable tool for providing effective genetic counselling and preventive actions for these families.
Materials and methods: To assess the abnormal splicing of twenty UVs identified in *BRCA1* and *BRCA2* genes in families from the South Catalonia region, a combination of several methods has been used, including *in silico* prediction tools to assess they putative effect and mRNA analysis. A comparison of the mRNA results and splicing prediction bioinformatic methods has also been performed.
Results: Four of the variants detected have been previously reported. Two variants of *BRCA1* gene (c.331+1G>A, c.5397-1G>A) and one of *BRCA2* gene (c.744+2T>A) showed an abnormal splicing pattern also predicted by the bioinformatic programs, and they had been previously reported. The rest of the UVs analyzed did not show an abnormal splicing effect at RNA level. Of these 17 UVs all were novel except c.9729+9A>C (*BRCA2)* that had been previously reported.
Conclusions: Consistent with previous reports, our study shows that the combination of several *in silico* methods yields highly accurate information, making it a reliable tool for selecting those variants for sequencing mRNA analysis.

Table 1. Bioinformatic Prediction Scores and In Vitro Splicing Results of BRCA1 and BRCA2 Unclassified Variants

Variant	Site[a]	Human Splicing Finder [0-100]		MaxEntScan [0-12]		NNsplice [0-1]		GeneSplicer [0-15]		SSF [0-100]		Interpretation based on bioinformatic prediction tools	In vitro splicing result
		Variant score	Wild type score	Variant score	Wild type score	Variant score	Wild type score	Variant score	Wild type score	Variant score	Wild type score		
BRCA1 (NM_007294.3)													
c.212+1G>A	D	0 (-100%)	78,1	0 (-100%)	7,8	0 (-100%)	0,9	0 (-100%)	2,8	0 (-100%)	77,4	Interruption of intron-exon junction	exon 5 deletion
c.302-24_302-22del	A	—	—	—	—	—	—	10 (+19%)	8,4	—	—	No changes predicted	wild type
c.417A>G	D	73 (+7,2%)	68,1	—	—	-	—	—	—	—	—	No changes predicted	wild type
	A	0 (-100%)	83,3	—	—	—	—	—	—	—	—	Interruption of intron-exon junction	
	A	75,2 (+1,2%)	74,3	—	—	—	—	—	—	—	—	No changes predicted	
c.4676-10ST>C	—	—	—	—	—	—	—	—	—	—	—	No changes predicted	wild type
c.5278-1G>A	A	0 (-100%)	93,6	0 (-100%)	13,1	0 (-100%)	1	0 (-100%)	14,4	0 (-100%)	90	Interruption of intron-exon junction	7 bp deletion exon 21
BRCA2 (NM_000059.3)													
c.67+62T>G	—	—	—	—	—	—	—	—	—	—	—	No changes predicted	
c.516+14C>T	D	67,3 (-2,9%)	69,3	—	—	—	—	—	—	—	—	No changes predicted	wild type
c.516+2T>A	D	0 (-100%)	86,9	0 (-100%)	8,9	0 (-100%)	1	—	—	0 (-100%)	87,5	Interruption of intron-exon junction	exon 5 deletion
c.556G>C	A	0 (-100%)	66,5									Interruption of intron-exon junction	wild type
c.680C>T	D	—	—	8,3 (-3,5%)	8,6	0,9	0,9	—	—	75,8 (+0,5%)	75,4	No changes predicted	wild type
c.6928A>C	A	69.9 (-0.1%)	70	—	—	—	—	—	—	—	—	No changes predicted	wild type
c.7007+22_7007+23del	A	—	—	4.1 (-6.8%)	4.4	—	—	—	—	—	—	No changes predicted	wild type
c.8023A>G	D	84,4	—	9	—	0,9	—	—	—	82,3	—	Creation of new donor site	wild type
c.8854A>G	—	—	—	—	—	—	—	—	—	—	—	No changes predicted	wild type
c.8850G>T	A	0 (-100%)	67,6	—	—	—	—	—	—	—	—	Interruption of intron-exon junction	wild type
c.9116C>T	D	—	—	2,4 (-44,2%)	4,3	0 (-100%)	0,6	—	—	—	—	Interruption of intron-exon junction	wild type
c.9501+9A>C	D	73,2 (-10,7%)	82	0,9 (-30,8%)	1,3	—	—	2,8 (+75%)	1,6	0 (-100%)	77,1	Disruption of intron-exon junction	wild type
	A	89 (+2,3%)	87	2,3 (+95,7%)	0,1	—	—	—	—	—	—	New cryptic acceptor site activation	wild type
c.10234A>G	A	74,7	0	—	—	—	—	—	—	—	—	New cryptic acceptor site activation	wild type

Bracketed percentages refer to the difference between variant and wild type scores.
[a] D donor site, A acceptor site, — no predicted donor or acceptor site

J12.53
Familial adenomatous polyposis: a regional audit of families and recommendations

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We have recently audited our families with familial adenomatous polyposis (FAP) identified from positive diagnostic or predictive tests for pathogenic *APC* mutations performed by our laboratory over three decades. Our service does not currently have a formally maintained register of FAP families. Genetics patient records were obtained and reviewed. A total of 183 patients from 42 families were ascertained. Further data on demographics will be presented. Management was audited against national and international guidelines. The majority (94.0%) of patients had recorded pre-test counselling. There was no formal written consent for genetic testing documented for 31.7% of patients, however, this has improved in the most recent 5-year cohort (90.5% documented). Deficits were evident in recording of screening recommendations, although generally improving over time. Formal recording in the notes of upper gastrointestinal screening recommendations was particularly low (42.6%). Presentation of the audit results in the department has highlighted the benefit of documenting consent. Standard letters and leaflets have been updated to improve advice and documentation. The audit identified 30 families with at-risk first-degree relatives who were not recorded as having been seen in our service. Letters are being sent to patients to remind them who in the family is at risk and regarding the availability of genetic testing, if not already performed. The audit demonstrated better recording of practice over time, but also areas for further improvement using simple measures.

J12.54
Lamin A levels in the metastasis potential of lung adenocarcinoma cells from pleural effusions

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The type V intermediate filament lamins are the principal components of the nuclear matrix, including the nuclear lamina. Lamins are divided into A- and B-types. The alternative splicing of LMNA produces two major A-type lamins, lamin A and lamin C. A-type lamins have been suggested as biomarkers for cancer diagnosis, prognosis and/or follow-up. The aim of the present study was to investigate lamins in cancer cells from metastatic pleural effusions using immunofluorescence, western blotting and flow cytometry. In a sub-group of lung adenocarcinomas, we reported reduced expression of lamin A but not of lamin C that cannot be explained by miR-9-induced degradation of lamin A mRNA. Moreover, the reduction in lamin A expression

was correlated with the loss of epithelial membrane antigen (EMA)/MUC-1, an epithelial marker that is involved in the epithelial to mesenchymal transition (EMT). Finally, the decrease in lamin A expression was correlated with an increased number of lung adenocarcinoma metastatic sites, with a preferential localization in bone.
We also hypothesized that lamin A expression could be regulated by other mechanisms, such as SRSF1, a LMNA splicing factor, or MALAT-1, a long, non-coding nuclear RNA, which negatively controls miR-9 expression and is implicated in SRSF1 regulation. Both SRSF1 and MALAT-1 are known to be involved in metastasis development in lung cancer.
In conclusion, low lamin A but not lamin C expression in pleural metastatic cells could indicate the lung origin of pleural metastatic adenocarcinomas and could represent a pejorative marker associated with the EMT and metastatic potential.
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J12.55
Molecular classification of colorectal cancer with/without peritoneal carcinomatosis by gene expression signature

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Aim. Colorectal cancer (CRC) is a heterogeneous disease. CRC with peritoneal carcinomatosis (PC) is most unfavorable prognosis group. Molecular classification of CRC (Sadanandam et al., 2013) defined five distinct molecular subtypes based on the preferentially expressed genes: stem-like, SFRP2; transit-amplifying, CFTR; goblet-like, MUC2 and TFF3; enterocyte, MUC2 without TFF3; inflammatory, RARRES3. CRC groups have differences in prognosis and vary in degree of response to chemotherapy.
Methods. We analyzed 27 tumors obtained from patients with PC and 45 stage I-III CRC, among last ones six samples were obtained from patients with Lynch syndrome (LS). Subtypes classification was validated by analyzing 6-gene expression signature by RT-PCR. KRAS /BRAF mutations were analyzed by sequencing.
Results. The various subtypes frequency in CRC with PC and stage I-III was: stem-like - 44.5% (12/27) and 15.6% (7/45); transit-amplifying - 37% (10/27) and 57.8% (26/45); goblet-like subtype - 3.7% (1/27) and 13.3% (6/45); enterocyte - 7.4% (2/27) and 4.4% (2/45); inflammatory - 7.4% (2/27) and 8.9% (4/45), respectively. LS-tumors were: 3 - inflammatory; 2 - goblet-like and 1 - transit-amplifying subtypes. KRAS/BRAF-mutations were detected in 70.4% CRC with PC (19/27) and 46.7% (21/45) in stage I-III CRC. Four from 5 BRAF-V600E mutations was found in stem-like subtype.
Conclusion. The prevalence stem-like subtype in CRC with PC and its association with BRAF-mutation confers aggressiveness, high metastatic potential and poor prognosis of this subtype. LS-tumors belonged to more favorable subtypes that confirm the markedly better prognosis for them. Molecular classification of CRC can serve for tumor subtype assessment suggesting specific treatment for each one.

J12.56
Frequency of the CHEK*2 1100delC among breast cancer patients in Iran

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Breast cancer is one of the most common cancers and the main cause of cancer-related deaths among women worldwide. The mutation in BRCA1 and BRCA2 genes are mainly involved in just 15% to 20% of all hereditary form of breast cancer but there is still a major proportion of hereditary breast cancer with no BRCA mutation detected. It has been proposed that a significant number of breast cancers are caused by defects in larger number of moderate or low penetrance genes. CHEK2 is a third high breast cancer susceptibility gene with low penetrance alleles that has been shown to best explain residual non-BRCA aggregation of breast cancer. CHEK2 acts as a tumor suppressor gene that directly regulates the functions of p53 and BRCA1 in response to DNA breakage which stopped cell proliferation and initiates DNA repair.
In this study we attempted to investigate the frequency of 1100delC mutation in CHEK2 gene in breast patient to improve screening strategy in Iran and provide genetic testing and counseling to family members of the patients.
Breast cancer (N=50) and control samples (N=50) which were all pathologically diagnosed, were screened for the 1100delC mutation by RFLP-PCR method. In all mutation-positive cases, results were confirmed by sequencing. None of our 100 samples who underwent genetic testing for 1100delC mutations had the *CHEK2**1100delC mutation. Our preliminary data suggest that screening for CHEK2*1100delC mutation in Iranian patients is not warranted.

J12.57
Non-invasive early detection of colorectal cancer: Determination of methylation pattern of ALX4 gene promoter in plasma

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Background: To develop a non-invasive screening method for colorectal cancer, we evaluated the methylation of ALX4 gene promoter in serum samples from patients with colorectal cancer (CRC) and equal number of healthy individuals. Materials and Methods: In serum samples from 25 patients with colorectal cancer and 25 healthy control subjects, isolated serum free-floating DNA was treated with sodium bisulfite and analyzed by methylation-specific polymerase chain reaction (MSP) with primers specific for methylated or unmethylated promoter CpG island sequences of the ALX4 gene. Results: Methylation of the ALX4 gene promoter was present in the serum DNA of patients with adenoma and colorectal cancer. A sensitivity of 68% and specificity of 88% were achieved in the detection of promoter methylation in colorectal neoplasia samples. The difference in methylation status of the ALX4 promoter between the patients with colorectal neoplasia and the control group was statistically highly significant ($P < 0.001$). Conclusions: The results indicate that this serum free DNA test of methylation of the ALX4 gene promoter is a sensitive and specific method. Therefore in combination with other useful markers it seems ALX4 has the potential of a clinically useful test for the early detection of colorectal cancer.
Key Words: Colorectal cancer, DNA methylation, Free-floating DNA, non-invasive colorectal cancer diagnosis

J12.58
New epigenetic markers of minimal residual disease in pediatric acute myeloid leukemia identified by unbiased screening of differential methylation of the genomes

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Introduction: Malignant neoplasms are characterized by genetic heterogeneity. Still there is a class of molecular genetic changes which characterizes all malignant tumors, namely, abnormal DNA methylation. The aim of our investigation was to develop a system of molecular genetic markers of minimal residual disease (MRD) in pediatric acute myeloid leukemia (AML) based on abnormal methylation of certain genomic loci.
Materials and Methods: The study involves samples of biological material of the bone marrow from AML patients and donor bone marrow used for transplantation. Identification of aberrant methylation is carried out by an unbiased DNA differential methylation screening method developed within this study. Experimental design has been carried out with the «AIMS in silico» software package developed in our laboratory.
Results: We have identified 16 novel genomic loci abnormally methylated in pediatric AML (15 belonging to the promoter CpG islands and one belonging to an intergenic CpG island on 7p21.1). Two of the genes (RXRA and KHSRP) with abnormally methylated promoters encode proteins involved in the epigenetic regulation of gene expression. We propose a system of 13 DNA methylation markers (belonging to the promoter regions of EGFLAM, RXRA, MAFA, TMEM176A /TMEM176B, KHSRP, TMEM200B, ABCG4, GSG1L, CLDN7, CXCL14, DLK2, AIFM3 and SOX8) for determination of the MRD in pediatric AML.
Conclusion: We have developed a panel of molecular genetic markers and an original technique for the assessment of aberrant DNA methylation in AML in children.
This study was supported by a grant from the Russian Foundation for Basic Research (research project 14-04-32295).

J12.59
Investigation of the role of microRNA biogenesis gene polymorphisms in prostate cancer development

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Prostate cancer (PCa) is one of the most serious oncological diseases in men with an incidence higher than that of all other solid tumors. Currently, it is the second cause of cancer mortality worldwide. The aberrant expression of microRNAs (miRNAs), small non-coding RNAs that negatively regulate gene expression, is related to the development of several cancers, including PCa. Since miRNAs serve as phenotypic signatures of different cancers, they appear as potential diagnostic, prognostic and therapeutic tools.

To determine whether miRNA polymorphisms are associated with PC risk we examined 30 SNPs of 18 microRNAs biogenesis genes *(GPC1, FAM212B, DDX20, FAM57A, DROSHA, C5orf22, AGO1, AGO2, RAN, PIWIL1, DICER1, GEMIN4, DGCR8, MIR196A2, NSRP1, MIR27A, DDX5),* using the OpenArray genotyping technology, in PCa patients of Russians (N=141), Tatars (N=69) and Bashkirs (N=52) and matched controls (N=95, N=137, N=56, respectively) from Russia.

We evaluated the association between SNPs in the mentioned genes and PCa risk as well as clinical characteristics (PSA level, clinical and pathological stage) in men (266 patients who underwent a prostatectomy and orchectomy) using logistic regression.

We observed that in Russians rs563002 (*G/*G vs. *A/*G+*A/*A: OR=0.21, CI=0.08-0.53), rs1057035 in DICER1 (*G/*G vs. *A/*G+*A/*A: OR=0.35, CI=0.13-0.88), rs1640299 in DGCR8 (*T/*T vs. *T/*G+*G/*G: OR=0.49, CI=0.26-0.92) were associated with decreased PCa risk and rs1640299 in DGCR8 (*G/*G vs. *T/*G+*T/*T: OR=4.48, CI=1.42-15.77) was associated with increased PCa risk. In Bashkirs rs595055 in *AGO1* was associated with increased PCa risk*:* (*T/*T vs. *C/*T+*C/*C: OR=2.48, CI=0.98-6.32).

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J12.60

Diagnostic and prognostic value of IDH1 mutation in glioblastoma patients

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Glioblastoma multiforme (GBM) is the most common and aggressive primary brain tumor in adults, with a poor prognosis because of its resistance to radiotherapy and chemotherapy. The GBM patient survival time of approximately 1 year necessitates the identification of novel molecular targets and more effective therapeutics. Mutations in the gene isocitrate dehydrogenase 1 (IDH1) are present in up to 86% of grade II and III gliomas and secondary glioblastoma, and could be used as a biomarker for this subset of gliomas.

The purpose of this study was to investigate the incidence of mutations in IDH1 and IDH2 genes in patients with glioblastoma multiforme from Vola-Ural region of Russia and establishing correlation mutations in these genes with the treatment and survival.

The studied groups included 15 patients with glioblastoma multiforme. Genomic DNA was extracted from tumor sample and peripheral blood leukocytes by standard phenol/chloroform method. Mutation status was studied by direct sequencing of IDH1 and IDH2 genes.

As a result of screening for mutations in the IDH1 gene mutation G395A was discovered in 5 of 15 patients with GBM, which leads to the substitution of adenine for guanine at position 395 (p.R132H). The frequency of this mutation in our study was 33%. Mutations in IDH2 gene were not found.

We confirmed that the survival rate of patients with IDH1 mutations 2-fold higher than those without mutation. In addition, patients with mutations in the IDH1gene had a favorable response to treatment with temozolomide, which indicates about the importance of screening for this mutation in GBM patients.

J12.61

Spectrum of EGFR mutations in Bulgarian patients with non-small cell lung carcinoma

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Mutations in epidermal growth factor receptor (EGFR) are considered as predictive biomarker for the clinical and radiographic responses to tyrosine kinase inhibitors (TKIs) in the treatment of non-small-cell lung cancer (NSCLC).

The most frequent activating mutations are inframe deletions in exon 19 and L858R in exon 21. These two mutations comprise about 85% of all EGFR mutations. Other EGFR mutations and multiple EGFR mutations in one tumor have not been comletely characterized in Bulgarian patients. In order to establish the spectrum of EGFR mutations in Bulgarian patients with NSCLC we investigated total of 294 tumors using qRT PCR techniques.

The morphology of all 294 cases included 51.7% adenocarcinomas, 23.5% squamous cell carcinomas, 1.3% adenosquamous and 22.8 % other types.

Activating mutations in EGFR were found in 9% (25) of patients. Among all mutations the most frequent were a deletion in exon 19 (52%), followed by mutation L858R in exon 21 (24%). In 1 male patient (4%) a compound EGFR mutation (S768I in exon 20 and G719X in exon 18) was found.

Evaluation of EGFR status is important because TKIs are effective in patients whose tumors harbor activating mutations in the tyrosine kinase domain of the gene. We found low frequency of compound EGFR mutations in our patients.

J12.62

Pathways associated with relapse and high risk in childhood acute lymphoblastic leukemia

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Introduction: Relapsed acute lymphoblastic leukemia (ALL) is one of the leading causes of death among children with cancer and the prognosis for early relapse remains poor. To discover the underlying pathways that may play a role in resistance, we analyzed gene-expression profiles in 818 ALL samples.

Materials and Methods: We performed gene-expression profiles in 16 matched diagnosis/ early relapse childhood ALL pairs, flow-sorted normal B cell progenitor subpopulations and CD4+CD8+ T cell by using the Illumina HumanHT-12v4 ExpressionChip. To extend the data, genome-wide expression data was retrieved from eleven ALL datasets via the Gene Expression Omnibus (GEO). The dataset contained expression data for 618 diagnosed,168 relapsed ALL samples and nine healthy controls. Data analysis was carried out in the R environment.

Results: Matched diagnosis/early relapse ALL samples clustered closely together and limited gene showed significant differences that involved in cell cycle regulation and apoptosis. Probe sets -1343 total were identified significantly different at diagnose who had not shown relapse compared to diagnose samples that relapsed in the future. Interestingly, various transcription factors had been down regulated in relapse-diagnose samples. ALL subgroups also compared with their controls. Leukemia samples showed aberrant expression of transcription factors that involved with MAPK and cell lineage pathways.

Conclusions: We have identified potential genes and pathways at relapse and initial diagnose that may play a direct role in drug resistance and leukemia development in childhood ALL. Ongoing further validation of the functional role of some of these genes will contribute the enlightenment of leukemogenesis.

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J12.63

Copy number characteristics of the 10q26.3 chromosome region containing the MGMT gene in glioblastoma

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Inactivation of the MGMT gene located at 10q26.3 predicts glioblastoma's sensitivity to alkylating agents. Methylation of the MGMT gene promoter is considered to be the key mechanism for this gene silencing and the marker of a favorable response to alkylating drugs. Deletion can present the alternative mechanism of MGMT gene inactivation. Previously we have been the first to conduct a targeted analysis of loss of heterozygosity (LOH) at 10q26.3 and have shown LOH at the MGMT region in 63.2% (74/117) glioblastoma samples. However LOH merely reflects allelic imbalance in the area without detailed information on the gene copy number. In order to assess copy number alterations at the 10q26.3 region in glioblastoma samples with identified LOH, we have developed a system for quantitative microsa-

tellite analysis (QuMA). QuMA is based on amplification of microsatellite loci that contain (CA)n repeats where the repeat itself is the target for hybridization by the fluorescently labeled probe. The reference pool contains primer pairs for six genomic regions located on different chromosomes in which copy number violations are not typical for glioblastoma. In 51.5% (34/66) of the samples only one copy of the tested locus was found (deletion), while in 48.5% (32/66) two copies were detected (acquired uniparental disomy, aUPD). Thus, we have shown that MGMT LOH in glioblastoma can reflect either a deletion or an aUPD. The deletion of MGMT gene requires detailed study as a potential marker of glioblastoma sensitivity to alkylating agents.
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J12.64
Implementation of rapid, routine BRCA gene testing for ovarian cancer patients

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Background
~15% of ovarian cancer (OC) patients have germline BRCA mutations. Knowledge of mutation status increasingly impacts treatment e.g. eligibility for PARP inhibitors. It is also important for ongoing patient management and provides risk information for relatives. In most European countries including UK, OC patients have restricted access to BRCA testing, despite meeting testing eligibility.
Methods
We developed an 'oncogenetic' pathway in which consent for BRCA gene testing in OC patients was undertaken by cancer team members that had completed 30 minute online training. Clear, interpreted results were returned by Genetics together with an appointment for all individuals with mutations. Cascade testing to relatives was performed through Genetics.
Results
207 OC patients were offered testing in 18 months and all accepted. Average time to result was 4-6 weeks. The BRCA result impacted management of 133 patients. Mutations were identified in 33 patients (16%); only 16 had a family history of breast or ovarian cancer. All mutation-positive women were seen in Genetics and ~100 relatives have been referred for counselling and consideration of predictive testing. Patient and clinician feedback was extremely positive; >95% of patients found the pathway simple and effective.
Conclusions
The Oncogenetic model allows flexible, patient-centred, high-throughput gene testing for OC patients with considerable time and cost savings compared to traditional models, and higher mutation detection rates. It is now the standard pathway for BRCA testing in OC patients at the Royal Marsden hospital. This work was undertaken by the MCG programme (www.mcgprogramme.com) funded by the Wellcome Trust Grant 098518/Z/12/Z.

J12.65
The differential gene expression in clear cell renal cell carcinoma and biomarker development.

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With the aim to receive additional information on mechanism of clear cell renal cell carcinoma (ccRCC) progression and development biomarkers we studied two groups of genes. The first was made up of genes with the most expression. By bioinformatic analysis of gene expression databases 200 genes were selected. After quantitative RT-PCR of ccRCC sample in comparison with normal renal tissue 21 genes with the most frequent increased expression were identified. The majority of these genes were direct targets of HIF1α. It was found an association of six gene decreased expression (CA9, NDUFA4L2, VWF, IGFBP3, EGLN3, BHLHE41) with overall survival (OS) and genes VWF, IGFBP3,STC2 with metastasis (p = 0,008 - 0,044). Among these genes were as novel, without established significance in ccRCC, as NDUFA4L2 (OR = 0,048; 95% CI: 0,005 - 0,444), so well known biomarkers, as CA9. The second group was formed of genes participate in angiogenesis (5 genes), PI4K-AKT-mTOR pathway (10 genes) and EGFR. Only VEGFR1 and VEGFA decreased expression were interconnected with both OS and metastasis (p = 0,009 - 0,023). Increased expression in significant association with OS was observed for SAA1 and three genes - CSF1R, Fn1 and C1QA tended to be increased. All these genes are associated with inflammatory response. The data give a possibility for proposition of consistent involvement HIF1A -> HIF2A -> NF-kB/STATs modules, which can lead to the development of metastasis and reduced OS. Thus, for the first time shown coordinated expression decreasing of number of regulated HIF1α genes in connection with OS and metastasis.

J12.66
Allelic imbalance and epigenetic changes as size markers of field cancerization in non-small cell lung cancer

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Non-small cell lung cancer (NSCLC) is characterized by multiple genetic alterations such as loss of heterozygosity (LOH), microsatellite instability (MSI), promoter hypermethylation and changes of miRNA expression. According to a field cancerization (FC) phenomenon the adjacent histologically normal tissue plays an important role in tumor progression by triggering the transformation process.
The aim of the study was the analysis of genetic alterations in tumor and adjacent tissue to determine the FC size and to reveal associations with clinico-morphological features of patients.
The study group included 135 patients with NSCLC. From each patient 4 FFPE samples were analyzed: tumor, adjacent normal lung tissue at 2, 5, 10 cm. LOH/MSI analysis was evaluated by PCR using 7 microsatellite loci. Promoter hypermethylation in genes *RASSF1A FHIT, DAPK1, CDH1, CD44, TIMP3, MGMT* was investigated by methyl-sensitive PCR. The expression levels of miRNAs let-7a, miR-155, miR-205 were measured by real-time PCR.
Our results demonstrated that LOH/MSI occurs only in tumor while promoter hypermethylation occurs also in adjacent tissue at 2, 5 cm, but not at 10 cm. The downregulation of let-7a, miR-155 in adjacent tissue is lower than in tumor. The levels of investigated miRNAs in adjacent tissue vary depending on tumor differentiation - in patients with differentiated tumors it is higher than in the group with poorly differentiated tumors.
We postulate that FC size in NSCLC is at least 5 cm from tumor and includes only epigenetic but not structural (LOH/MSI) alterations. The evaluation of epigenetic changes in adjacent tissue (e.g., surgical margins) can potentially be used for postsurgical prognosis.

J12.67
IDH1/2 mutations and concurrent DNA methylation in meningiomas

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Background. IDH mutations are linked to an altered methylome phenotype in gliomas, involving key genes participating in cell growth, development, cell cycle and metabolism. Our aim was to search for IDH1/2 mutations in correlation with promoter methylation for MGMT, APC, PTEN, RASSF1, DAPK1 and MLH1 genes in an effort to delineate their possible role in the pathogenesis of meningiomas.
Methods: 75 meningiomas were examined (grade I n=42, grade II n=26, grade III n=6). Methylation and mutation status were assessed by HRM, pyrosequencing/or Sanger sequencing.
Results. IDH1 mutations at codon 132 were observed in 5% of the cases, with only 1 case bearing the hot-spot mutation p.R132H. IDH2 mutations were recorded in three cases including two silent mutations, (p.D117V, p.R172R, p.L143L). A varying level of promoter methylation for APC, RASSF1A, MGMT, DAPK, hMLH1 was detected in 37%, 25%, 41%, 41%, 28% of the cases respectively. 75.5% of the samples showed methylation at one or more promoters, in 14%, 30%, 22%, 8% of the cases for one, two, three or four promoters respectively. All IDH1/2 mutant samples displayed concurrent methylation of two or three promoters. No statistical associations were elicited between methylation, mutations and grade.
Conclusion. This report is the first to analyse simultaneously the presence of IDH1/2 mutations along with methylation status of five promoters in a cohort of meningioma samples of different grades. A subgroup of meningiomas presented IDH1/2 mutations along with partial promoter methylation, suggesting a possible role in their pathogenesis.

J12.68
Breast cancer in female Peutz-Jeghers syndrome patients:

risk assessment in a large patient cohort and surveillance recommendations

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Female Peutz-Jeghers syndrome (PJS) patients have a highly increased cancer risk, including breast cancer (BC). Risk estimates for BC in these patients vary, while information on clinical and histological characteristics is scarce. In the present study, we assessed the BC risk and clinicopathological features of BC in a large PJS cohort, and sought to formulate a present-day BC surveillance recommendation. Cases were identified from the Dutch PJS cohort. PJS was diagnosed according to international criteria. Clinical data were collected and radiological and histological data were reviewed. Cumulative BC risks were calculated by Kaplan-Meier analysis and relative risk by Poisson regression analysis. Of 145 PJS patients, 75 (52%) were female. Nine women from 8 families were diagnosed with BC at a median age of 50 years (range 34-61). Mammography allowed good visibility of all but one BC. The majority of
analysed BCs was of good or intermediate differentiation grade and all invasive tumours were hormone sensitive and Her2-negative. Cumulative BC risk was 62% (95% CI 31%-93%) at age 65, and relative risk was 6 (95% CI 3 to 13, p<0.001) compared to the age matched general population. BC risk for female PJS patients is highly increased, approaching that of BRCA-mutation carriers, while PJS-associated BCs seem to have a later onset and more favourable clinicopathological characteristics than BRCA-mutation carriers. We propose to start annual BC surveillance with mammography in female PJS patients as of 30 years, with additional MRI in patients with dense breast tissue. Prospective evaluation of this schedule is required to determine its effectiveness.

J12.69

Association between single nucleotide polymorphisms in candidate genes or regions and ovarian cancer risk for woman in Bashkortostan

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A genome-wide association study recently identified an association between single-nucleotide polymorphisms rs3814113 at 9p22.2, rs8170 at *MERIT40* gene, rs2072590 at 2q31, rs2665390 at *TiPARP* gene, rs10088218 at 8q24, rs9303542 at *SKAP1* gene, rs2736108 and rs2736109 at *TERT* gene and risk of ovarian cancer.
We analyzed the association this polymorphisms and ovarian cancer risk in cases (n=227) and controls (n=373) from Bashkortostan. Single-nucleotide polymorphisms detection was performed by allelic discrimination *Taqman*.
We have identified an association polymorphism rs2072590 (2q31) with risk of ovarian cancer for women in the general population, OR = 1.5, 95% CI 1.0-2.1, p = 0.045. In different ethnic groups the patient and control genotype and allele frequencies were not significantly different. Polymorphism rs2072590 is localized in a domain containing homeobox genes required for the regulation of embryogenesis and organogenesis in the noncoding region between *HOXD3* and *HOXD1* genes. Both genes involved in tumorigenesis.
Also we identified an association polymorphism rs8170 in *MERIT40* gene with the risk of ovarian cancer in Russian, OR = 0.5, 95% CI 0.3-0.9, p = 0.03. MERIT40 is necessary for BRCA1 localization to DNA damage sites.
Our data indicate that rs2072590 and rs8170 polymorphisms are associated this ovarian cancer risk for woman in Bashkortostan.
This work was supported by the Russian Foundation for Basic Research (14-04-31169) and State task of the Ministry of Education and Science of the Russian Federation (2985).

J12.70

Methylation profiling of Socs1 gene in colon neoplasms

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Background: Suppressor of cytokine signaling 1 (*Socs1*) protein plays an essential role in inhibition of JAK-STAT pathway, which is important for differentiation, proliferation and apoptosis of cells involved in tumorogenesis. Hypermethylation of the promoter CpG islands in the *Socs1* gene is an epigenetic phenomenon associated with gene silencing that leads to inactivation of this tumor suppressor gene function in colorectal cancer (CRC).The aim of this study was to determine the methylation status of *Socs1* gene promoter in colorectal neoplasms.
Materials and Methods: We investigated the aberrant methylation status of *Socs1* gene in 37 polyp and 13 tumor biopsies obtained from participants (24 female and 26 male with age of 53±26) and 50 corresponding nonmalignant epithelium, by methylation specific PCR (MS-PCR). Tissue samples were collected during 2011 to 2013, after colonoscopy from participants. Informed consent was obtained from patients.
Results: Eighteen percent (8 of 50) of the studied biosies were found to have methylated CpG islands in *Socs1* gene promoter region.There was a significant association between promoter methylation and size of neoplasms. Frequency of promoter methylation was higher in smaller neoplasms under 10 mm in size. None of the nonmalignant epithelium samples were methylated in the promoter region.
Concussion: Detection of *Socs1* promoter methylation in colon neoplasms may provide an informative approach for early detection of CRC.
Key words:
Socs1, Methylation, MSP, Colon neoplasm

J12.71

Phenotype of MUTYH mutation carriers: Surprise, surprise!

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The autosomal recessively-inherited form of colorectal adenomatosis polyposis (FAP2) results from homozygous or compound heterozygous mutations in the MUTYH gene. MUTYH, the human homologue of the E.coli mutY gene, is involved in base excision repair and cellular response to oxidative DNA damage. Two founder mutations (c.536A>G and c.1187G>A) account for the majority of pathogenic mutations reported to date. Carriers of two mutated alleles, who generally have onset of „attenuated polyposis“ as young adults, have a high risk of colorectal cancer, as well as some increase in the incidence of duodenal, gastric, endometrial and breast cancers. Data regarding polyp and cancer risk in monoallelic mutation carriers are limited and conflicting, with some evidence for an increased incidence of colorectal and breast cancers. In addition, until the recent inclusion of MUTYH in multigene cancer-risk panels offered on a clinical basis, little was known of other mutations, hence the high rate of Variants of Unknown Significance (VUS) in clnical testing. Our experience with mutations and variants in the MUTYH gene (see selected cases in Table 1) suggests that cancer risk has been underestimated in monoallelic carriers, and that it may be appropriate to follow individuals with one or two MUTYH mutations as we do individuals with Lynch syndrome (Hereditary Non-Polyposis Colorectal Cancer, HNPCC).

Phenotype and Genotype: Selected Cases

HK; 33; M	personal cancer	adenoCA cecum	hetero for VUS pre-exon 11	c.934-2A>G
DI; 31; M	personal & family cancer	adenoCA rectum	hetero for VUS, exon 8	c.667A>G
CO; 62,F	polyps & cancers	lymphoma; endometrial adenoCA	hetero for mutation, exon 6	c. 536A>G
PA; 55; F	personal & family cancer	breast DCIS	hetero for mutation, exon 6	c.536A>G
RC; 46; M	attenuated polyposis coli	no malignancies	homozyzous mutations, exons 6 & 13	c.536A>G; c.11867G>A

J12.72

Genomic Variations detected by MLPA in Brazilian patients with Colorectal Cancer

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Colorectal cancer (CRC) is a health problem that is increasing in importance worldwide although its etiology is still poorly understood. Therefore we investigated copy number variation anomalies (CNVs) in genes related to colorectal cancer using MLPA technique (Multiplex ligation-dependent probe amplification) in 16 samples obtained from colorectal carcinoma. The total of 16 tissue samples were obtained by biopsy of 8 patients with CRC, being 8 neoplastic samples and 8 samples obtained from adjacent regions (10cm distant tumor tissue). The DNA was extracted using the QIAamp DNA Blood Midi Kit (QIAGEN, Valencia, California) and investigated by MLPA method (MRC-Holland®, Amsterdam, The Netherlands) with specific kits for the subtelomeric regions (P036 and P070). The results were analyzed using the software GeneMarker® (SoftGenetics, LLC, State College, PA - www.softgenetics.com).
Using MLPA technique we were able to identify several indels in many regions involving different genes. We detected genetic imbalances in 15 samples and observed a consistent pattern of distribution of genetic alterations (losses) in some genes as *KDM5A*(12p), *TNFRS18* (1p), *CTDP1* (18q),

*TRIML2(*4q*) IL17RA*(22q), *MTA1*(14q), *GAS8* (16q); and gains in *CHL1*(3p), *PSPC1*(13p*)*, *SOX12*(20p), *SYBL1*(Xq), *CDC16*(13q), *PDCD6*(5p), *RABL2B* (22q). Unexpected we observed genomic alterations in samples from adjacent regions of the tumor, suggesting that the adjacent regions are not the adequate to use as a control. MLPA emerges as an important tool to improve comprehension of genomic profile of CRC and also is a reliable and efficient method to measure aberrations in tumor genomes

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J12.73

Comprehensive genomic characterization of basal cell carcinoma

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Basal cell carcinoma (BCC) is the most common malignant neoplasm in humans; however, it has not been studied by international cancer consortiums. Most BCCs are caused by aberrant activation of the hedgehog pathway (SHH) by PTCH1 mutations. BCC's varying morphology and aggressiveness, as well as their response to SMO inhibitors, may be associated with the acquisition of secondary driver mutations.

To characterize the genomic landscape of BCC, we performed exome-sequencing of frozen material from 50 cases/matching germline samples and a validation cohort of 100 FFPEs. BCCs have ~88 SNVs/Mb; much higher than all other tumors, including melanoma. In all cases, primary drivers in PTCH1, SMO or TP53 were identified. As expected in tumors with high mutation rates, we observe secondary driver mutations in 55% of cases; remarkably, we detect additive activation of SHH downstream of SMO-GLI by MYCN (40% of cases), PPP6C (15%) and FBXW7 (6.5%) mutations. We experimentally determined that MYCN mutations promote N-Myc stabilization by impairing its ubiquitination by FBXW7. N-Myc stabilization, truncating FBXW7 mutations, and PPP6C p.R264C mutations found in our cohort are all known to stabilize AuroraA. We further show activation of the HIPPO pathway (30% of cases); we observe truncating mutations in PTPN14, involved in cytoplasmic sequestration of YAP1. Immunohistochemistry confirms the enrichment of nucleus-localized YAP1 in PTPN14-mutated tumors.

This study is the most comprehensive and integrative analysis of BCC to date. We provide new insights into mechanisms mediating resistance to SMO inhibitors via secondary oncogenic mutations and we report MYCN oncogenic point mutations for the first time.

J12.74

Germline mutations in MMR genes among Russian patients with Lynch syndrome

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Aim

Lynch syndrome is one of the most frequent inherited colorectal syndromes. The syndrome is caused by a mutation in one of the mismatch repair (MMR) genes: mainly MLH1, MSH2 and MSH6. The aim of this study was to study frequency and spectrum of germline mutations of MMR genes among Russian patients.

Methods

Microsatellite instability was studied in tumor samples of probands, which corresponded to next criteria: age ≤ 45 and/or family history of colorectal cancer. Five loci markers (NR21, NR24, NR27, BAT25 and BAT26) were analyzed by fragment analysis. Germline mutation in MLH1, MSH2 and MSH6 gene of patients with MSI-H (high level) were investigated by PCR, conformation-sensitive electrophoresis, Sanger sequencing and next generation sequencing.

Results

Microsatellite instability was found in 76 tumor samples. Of these 76 patients 24 (31.5%) had germline mutations in MMR genes. Fourteen germline mutations were found in MLH1, eight mutations - in MSH2 and two mutations - in MSH6. Eight of these mutations were nonsense, 6 - frameshift, 5 - splice sites and 5 - missense mutations.

Conlusion

Frequency of germline mutation in MLH1, MSH2 and MSH6 gene among present set of Russian patients is 58.33%, 33.33% and 8.33%, respectively.

J12.75

BRCA 1/2 mutation carriers in Reunion Island

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Introduction :

In Reunion Island, we have chosen to study the BRCA 1/2 mutation carriers, and thus to search for the presence of a founder mutation.

Methods :

This study is a retrospective, multicentric one, over 10 years, about 65 subjects, who are carrying a BRCA 1/2 mutation.

Their epidemiologic characteristics, as well as the all cancer and mutation characteristics, were analysed.

We have also compared the initial breast cancers of this population with the french national cohorte GENEPSO.

Results:

A specific mutation was found, the 2840C>A (exon 11) of the BRCA 2 gene, wich concerned 37 % of the Reunion's BRCA carriers. An usually BRAC 2 mutations number was also observed,

with 70 % of the subjects, against 35% of the GENEPSO cohorte.

Conclusion :

This particular BRCA mutations distribution is related to ethnic and cultural history of the Reunion Island, with a mixed population but compartmentalized in its sociodemographic functioning. Cancer's characteristics of this population appear to be usual, however this study shows the importance of hereditary risk factors cancer screening. It has to be based on a suggestive family history, but also on tumorous characteristics, like a triple negative profile before 50 years.

This study underlines the presence of a probable founder mutation, specific of Reunion Island.

We didn't found difference between Reunion or French BRCA mutation carriers, especially for the initial breast cancers, except younger age of occurence, less triple negative breast tumors, and a statistically significant high proportion of BRCA 2 gene mutations.

J12.76

NGS-assisted DNA-based digital PCR for a personalised approach to the detection and quantification of residual disease in CML patients

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Recent studies indicate that 40% of chronic myeloid leukaemia (CML) patients who achieve sustained undetectable BCR-ABL1 transcripts on tyrosine kinase inhibitor (TKI) therapy remain disease-free after drug discontinuation. In contrast, 60% experience return of detectable disease and have to re-start treatment, thus highlighting the need for an improved method of identifying patients with the lowest likelihood of relapse. Here we describe the validation of a personalised DNA-based digital PCR approach for quantifying very low levels of residual disease, which involves the rapid identification of BCR-ABL1 fusion junctions using targeted next generation sequencing coupled with the use of a digital PCR (dPCR) platform. BCR-ABL1 genomic breakpoints were successfully mapped in samples from 32 of 32 patients with early disease. Disease quantification by DNA-dPCR was performed using the Fluidigm BioMark platform on 46 follow-up samples from 6 of the 32 patients, including 36 samples that were in deep molecular remission (MR). Digital PCR detected persistent disease in 81% of MR samples, out-performing both RT-dPCR (25%) and DNA-based qPCR (19%). We conclude that dPCR for BCR-ABL1 DNA is the most sensitive available method of residual disease detection in CML and may prove useful in the management of TKI withdrawal.

J12.77

Down regulation of *SnoN/SKIL* gene in T-cell Acute Lymphoblastic Leukemia

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T-cell acute lymphoblastic leukemia (T-ALL), is a severe disease that occurs in the malignant transformation of T-cells in the thymus. Here we performed whole genome expression analysis in 31 T-ALL childhood patients. In addition to some well-known targets a new target has been identified in the patient group *SnoN/SKIL* gene, known as a regulator of TGF-β pathway, was down regulated when compared to thymocyte controls. Quantitative Real Time PCR and analysis of proteins by proximity ligation assay (PLA) also confirmed this down regulation in a larger patient group (n=72). In the thought of the reasons of this downregulation, we searched the possible miRNAs that target *SnoN/SKIL* gene via online databases. Among these miRNAs we select mir-223, which is also known to be deregulated in hematologic malignancies, and by Stemloop RT-PCR we found out that all the samples with low *SnoN/SKIL* expression show high mir-223 expression levels. When we block mir223 in Molt4 cell line, by anti-mir223 LNA *in vitro*, *SnoN/SKIL* expression was leveled up. In the same group when we checked the apoptosis levels by Annexin V flow cytometry assay we found out that when we block mir223, cells were lead to apoptosis almost 10 fold higher than untreated cells. Our study is still ongoing to validate the direct relation between *SnoN/SKIL* and mir223, but these preliminary results show that *SnoN/SKIL* gene, which is known to have dual role in different malignancies like being oncogene or tumor suppressor, might be acting as a tumor suppressor gene in T-ALL.
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J12.78

Next generation sequencing (NGS) of a 29-genes panel in individuals negative for BRCA1/2 testing.

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Introduction: BRCA1 and BRCA2 germline mutations account for 5-10% of breast-ovarian cancer families. A large majority of these familial aggregation remain unexplained. Recent studies report a 4-16% mutations prevalence when investigating other genes but BRCA1/2. New candidates are emerging. To further elucidate the role of these candidate genes in breast-ovarian cancer susceptibility, we selected a cohort of BRCA1/2 negative index-cases to be tested with a 29-genes panel.
Patients and methods: 24 affected women with at least 3 first degree relatives diagnosed with breast/ovarian cancers were recruited in 4 familial clinics in Alsace province (France). A first capture panel of 29 genes was designed. Genomic DNA Library preparation was performed using the SureSelect XT™ (Agilent) and sequenced with the MiSeq® Sequencing System (Illumina). Primary analyses were performed via the MiSeq Reporter Software, secondary analyses via an in-house bioinformatic pipeline.
Results: Sequencing (2x150bp) generated 7Gb of data, with 95% of the bases ≥QV30. All coding regions and intron-exon jonctions were covered (mean depth of coverage 150X. Preliminary results revealed 2 deleterious mutations: 1 frameshift on PALB2, and 1 nonsense mutation on RAD51C. Variants of uncertain significance will be reported. Further analyses will be needed to classify these variants.
Conclusion: This preliminary analysis support the contribution of PALB2 and RAD51C in breast/ovarian cancer families. Inclusion of new patients is currently ongoing.
Collection of such data together with clinical records are of importance to contribute to collaborative efforts to offer proper guidelines for breast and ovarian cancer risk management of mutation carriers.

J12.79

MicroRNA-330-3p functions as an oncogene in human esophageal cancer by targeting Programmed cell death 4

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MicroRNAs comprise a family of small non-coding RNA molecules that have emerged as key post-transcriptional regulators of gene expression. Aberrant miRNA expression has been linked to various human tumors. This study was aimed to identify novel miRNAs involved in the carcinogenesis of esophageal squamous cell carcinoma (ESCC) and their potential functions. We performed miRNA microarray and found that miR-330-3p was highly expressed in ESCC tumor tissues. Ectopic expression of miR-330-3p significantly promoted ESCC cell proliferation, survival, migration, invasion in vitro and stimulated tumor formation in nude mice. Knockdown miR-330-3p expression leaded to the opposite effects. Further, we revealed that miR-330-3p could repress the expression of programmed cell death 4 (PDCD4) by targeting its 3' un-translated region (UTR) and expression of PDCD4 was inversely associated with miR-330-3p in ESCC tissues. In addition, silencing of PDCD4 significantly promoted cell growth, cell migration, invasion and inhibited cisplatin-induced apoptosis. In conclusion, our findings demonstrated that miR-330-3p might play an oncogenic role in the development of ESCC partially via suppression of PDCD4 expression.

J12.80

Clonal hematopoiesis markers in Ph-negative myeloproliferative neoplasms (PH(-)MPN)

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Inroduction: In 2013 several researchers have published data describing recurrent mutations - insertions and deletions - in 9th exon of CALR gene among patients with Ph(-) MPN which allow to consider these mutations as a new marker of clonal hematopoiesis like JAK2 and MPL mutations. The goal of our study was to determine frequency of JAK2, MPL, CALR mutations in MPN patients and to analyze overall survival (OS) in groups with different clonal markers and triple-negative.
Methods: In our work we used PCR-RFLP, high resolution melting and direct sequencing methods. Results: JAK2V617F mutation was determine in 74/76(97,3%) pts with polycythemia vera (PV), 26/63(41,27%) pts with essential thrombocythemia (ET) and 21/43(48,83%) pts with primary myelofibrosis (PMF). 2/76(2,7%) pts with PV have mutations in 12th exon of JAK2. 515 codon mutations of MPL were observed in 3/43(6,97%) cases of PMF and 3/63(4,76%) cases of ET. Frequency of CALR mutations among pts with PMF and ET was 20,63%(13/63) and 13,95% (6/43) respectively. There was no reliable differences in OS in groups of pts with JAK2(+), MPL(+), CALR(+) and without mutations (p=0.127), but it should be mentioned that 4 of 6 died pts have unfavorable karyotype (+7, +8, del (5),(q13,q39), complex). It was not take into account when determining OS. Interestingly, OS in CALR-mutated group was 100% while OS in MPL(+) and triple-negative group was 67% and 80% respectively.
Conclusion: Genetic testing of clonal markers is insufficient. Only complex genetic and cytogenetic analysis of tumor cells has crucial importance for diagnosis and prognosis of MPN.

J13.01

Effect of Sodium benzoate preservative on micronucleus induction, chromosome break and Ala40Thr Superoxide dismutase gene mutation in lymphocytes

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Sodium benzoate is food preservative that inhibits microbial growth. The effect of sodium benzoate preservative on micronucleus induction, chromosome break and Ala40Thr Superoxide dismutase gene mutation in lymphocytes were studied. Sodium benzoate conc. 0.5, 1.0, 1.5 and 2.0 mg/ml were treated in lymphocyte cell line for 24 and 48 hrs. respectively. Micronucleus test, Standard chromosome culture technic, PCR and Automate sequencing technic were done for detect micronucleus, chromosome break and gene mutation. The results showed that at 24 and 48 hrs. incubation time, Sodium benzoate conc. 1.0, 1.5 and 2.0 mg/ml increased micronucleus formation when comparing with the control group ($P<0.05$). At 24 and 48 hrs. incubation time, Sodium benzoate conc. 2.0 mg/ml increased chromosome break when comparing with the control group ($P<0.05$). Sodium benzoate did not cause Ala40Thr in Superoxide dismutase gene. Sodium benzoate had the mutageneic and cytotoxic toxicity in lymphocytes caused by micronucleus formation and chromosome break.

J13.02

Balanced chromosomal rearrangements and their clinical significance

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The aim of the study is to present the correlation between the presence of chromosomal rearrangement (BCR) and the clinical impact on the carriers. During 2003 to 2014 2414 patient were investigated by conventional karyotyping. The retrospective study analyzed cases with BCR (reciprocal translocations, inversions and insertions) in relation to the original reason for referral, phenotype (any significant clinical findings) case type (prenatal, postnatal), sporadic/ familial inheritance. BCR were found in 44 out of 2414 (1,82%) studied diagnosed patients. These were 29 (65,9%) reciprocal translocations (one prenatal case with two translocations and one postnatal of complex tree way translocation included), 10 (22,7%) Robertsonian translocations and 5 (11,4%) inversions. A combination of numerical mosaicism and BCR was found in two different familial cases. The most common referral was reproductive problems: recurrent abortions in 21 (47,7%) and primary infertility in 5 (11,4%) followed by unbalanced offspring in 9 (20,4%). Abnormal phenotype with significant clinical findings (developmental delay/disability, behavioral abnormality, major psychosis, tuberous sclerosis, hypergonadotropic hypogonadism) was found in 6 (13,6%) of the studied patients. BCR were diagnoses mainly in patients with spontaneous pregnancy losses and primary infertility and in a less degree in families with unbalanced offspring or even phenotypes with some significant clinical findings. It is important to distinguish between truly balanced and those that appear balanced cytogenetically but are really unbalanced on molecular level . The concept of their difference is still arbitrary and subject to ongoing molecular investigations nowadays announced as "Saturation of the human genome with chromosomal breakpoints".

J13.03

The role of ROS in the context of chromosome aberrations induced by arecoline

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Backgrounds:Areca nuts are widely chewed in the southeast Asian. Arecoline is one of the major alkaloid in Areca nut. It has been evidenced arecoline-induced cytotoxicity in various mouse and human cell types, while the induced chromosome aberrations only in mouse cell lines but not in human cell lines. The arecoline-induced ROS (reactive oxygen species) was also identified in several human cell lines. It was known that the oxidative stress from the elevated ROS impairs cellular functions involving in cytotoxicity, cell cycle arrest, and DNA damage, etc. In this study, we would identify the role of ROS in the context of the arecoline-induced chromosome aberrations. Materials & Methods:The in vitro chromosome aberration assay was used to evaluate the extent of chromosome aberrations. The cell growth and cytotoxicity were assessed using MTT assay and PI staining. Apoptosis was analyzed using the Annexin-V/PI assay and TUNEL assay. The ROS was quantitated using DCFDA detector. Results:The result of cytotoxicity and chromosome aberrations (CAs) showed that CHO-K1 cells (Chinese hamster cell line) was more resistant to arecoline than S-G (human oral gingival epithelial cell line). The arecoline-induced CAs was dose dependent in both CHO-K1 and S-G cells. The curve of dose- CAs was slower in CHO-K1 than S-G cells. Surprisingly, we found that arecoline induced ROS in CHO-K1 cell, but no ROS in S-G cell. Conclusion:Based on these findings, we suggested that arecoline caused different toxic responses in different cell lines. The induced ROS attenuated the arecoline-induced cytotoxicity and CAs in CHO-K1 cells.

J13.04

Modulation of the expression of antioxidant enzymes and proinflammatory cytokines by cerium oxide nanoparticles in vitro.

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Action mechanisms of cerium oxide nanoparticles (nanoCeO$_2$) to the intracellular signaling pathways, including conditions of oxidative stress at present is not entirely clear. The mechanism of the antioxidant properties of nanoCeO$_2$ associated with the presence on the surface of cerium ions in different oxidation states (Ce3 + and Ce4 +). On the one hand, nanoCeO$_2$ can serve as free radical scavengers since they have mimetic activity as antioxidant enzyme activity, such as superoxide dismutase and catalase. On the other hand, there is evidence confirming the effect of nanoCeO$_2$ on intracellular signaling pathways.
We analyzed the expression levels of a wide range of target genes in human mesenchymal stem cells with combination of cerium dioxide nanoparticles (for 24 hours prior to irradiation at a dose of 10^{-7} M) and X-ray dose of 15 Gy by RT-PCR method. We showed that nanoCeO$_2$ synthesized by citrate method, can regulate the expression of some enzymes (MnSOD, CuSOD, catalase, IL-1, IL-6, TNF-a, iNOS), reducing the level of oxidative stress in the cell, thereby preventing the development of oxidative stress, induced by ionizing radiation.
These data allow us to propose the assumption that nanoCeO$_2$ can act not only as a scavenger of reactive oxygen species and to modulate the metabolic pathways functioning cells, causing the formation of a specific adaptive response.
The obtained data can serve as the basis for the development of new highly selective radioprotective for clinical radiotherapy.
The project was supported by grants from RFBR № 14-04-32199 mol_a and №14-44-03615 r_center_a

J13.05

Fetal anomaly and feto-placental discrepancy in karyotyping

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[Background] It is well known that confined placental mosaicism (CPM) is related to fetal growth restriction since chorionic villus sampling (CVS) has been becoming common. At our hospital, we experienced 4 cases of CPM in a few years and one of them had a hepatic tumor which is related to mosaicism. In this study, we focused on the frequency of CPM in the group of fetuses with anomalies not only fetal growth restriction.
[Method] In Japan, CVS is not so common and select more amniocentesis still now, so we have done the karyotyping of neonates and placenta (with all three embryonic cell lineages) postnatally. We obtained the samples from neonates with anomalies such as dismorphologic features, growth restriction and other problems including fetal death.
[Results] In 55 cases since November 2013 to date, two cases were failed at culture because of severe infection. Only 1 case was diagnosed as CPM and that case was clinically diagnosed as fetal growth restriction. This is 0.26% of FGR cases and 0.18% of total, while Kalousek et al. reported the prevalence of CPM detected at term in FGR could be between 8% and 60%.
[Conclusion] Against our hypothesis, fewer cases were diagnosed as CPM in this study. It suggests there might be some difficult point in diagnosing CPM at term pregnancy or the actual frequency of CPM would be rather low. We would like to continue this study with larger numbers to clarify the mechanism and the clinical impact of CPM.

J13.06

In vitro and in vivo preliminary genotoxicity study of dipotassium-tri oxohydroxytetrafluorotriborate K2[B3O3F4OH]

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Genotoxic effects of inorganic molecule dipotassium-trioxohydroxytetrafluorotriborate, K2[B3O3F4OH], recognized as a promising new therapeutic for the treatment of the epidermis changes, have been evaluated in this study. In vitro analysis included evaluation of genotoxic and cytotoxic potential of K2[B3O3F4OH] in concentrations of 0.01, 0.02, 0.05 and 0.06 mg/ml applying cytokinesis-block micronucleus cytome assay in human lymphocyte cultures of 4 volunteer peripheral blood donors. With the increase of K2[B3O3F4OH] concentration the frequency of micronuclei elevates as well but the differences are not significant. Also, there were no significant differences among the frequencies of nuclear buds and nucleoplasmic bridges in controls and treated cultures. NDI and NDCI values did not differ significantly, although the means of both values were slightly decreased in treated cultures in comparison to controls. In vivo genotoxic effects were analyzed on 4 mice of BALB/c strain per each group, applying reticulocytes micronucleus assay. K2[B3O3F4OH] in concentrations of 10, 20, 50 and 55 mg/kg have been administrated intraperitonealy to the animals. Significant decrease of reticulocytes ratio and increase of micronuclei frequencies against pre-

treatments have been found for both sampling periods of 48 and 72 hours only for the highest applied concentration of 55 mg/kg.

J13.07
Translocation t(18;19)(p11.2;p13.1) and its Clinical Implications; Case report and Mechanism of Pathogenesis

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A 30-year-old married female with one healthy child admitted to the medical genetics outpatient clinic for evaluation of recurrent fetal loss. Chromosomal analysis revealed a t(18;19)(p11.2;p13.1) translocation. Patient's parents, husband, and healthy child were also tested for chromosomal abnormalities. Married couple had a healthy 9-year-old child and 4 successive miscarriages, all at 5-6 weeks of pregnancy. First three abortions were spontaneous; the last was therapeutically induced . Family history revealed a sister with a known recurrent fetal loss and an early death of a male child from epileptic seizures at 6 years of age. Chromosomal analysis for the patient's sister, parents, healthy child and husband revealed t(18;19)(p11.2;p13.1) at patient's sister and mother. Chromosomal analysis of the patient revealed the same translocation. Questions concerning the probability of a patient to have a successful pregnancy and the risk of having an affected child were raised. With genetic counseling, patient was advised to have PGD and subsequent IVF to prevent the possibility of having an affected child or a miscarriage. IMPA2 gene and is responsible for a production of childhood febrile seizures, corresponds to 18p11.2 locus, which has been translocated to patient's chromosome 19. Although patient's sister's diseased epileptic child had no karyotyping, it is assumed that he had a chromosome with missing IMPase gene (chromosome 18 with a missing p11.2 locus), which caused decrease in IMPase production. Because of this occurrence, it is possible that the patient is also at risk of giving birth to a child with febrile epileptic seizures and migth result in early death of the child.

J13.08
Cytogenetic findings associated with X chromosome in the Genetic laboratory of Varna over 12 year period

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The cytogenetic analysis is still useful for the establishment of diagnosis in genetic diseases and in Eastern Europe it is basically the initial genetic analysis. The aim of this study is to reveal heterogeneity of X chromosome pathology among tested patients from the region of Varna, Eastern Bulgaria.
Patients and Methods: Retrospective analysis in the Laboratory of Medical Genetics was performed to find out patients with X chromosome abnormalities for a period of 12 years (2003-2014). These were cytogenetically examined phenotypic females and males at the age of a newborn to 53 years with indications: females with Turner-like phenotype, infertility (sterility or recurrent spontaneous abortions), premature ovarian insufficiency (primary or secondary amenorrhea), and ambiguous genitalia (congenital adrenal hyperplasia included). Conventional karyotyping with G-banding cytogenetic testing was performed.
Results: Significant X chromosomal aberrations were found in 57 (9.86 %) of all 578 indicated patients. The most common finding was Turner syndrome confirmed diagnosis with classical monosomy X in 13 (22.8%) or mosaic variants of numerical and/or structural abnormal cell lines of any known type 12 (21.0%) patients. Abnormal karyotype was found in 16(28.0%) (mostly females and one male) patients with infertility; 6 (10.5%) of females with premature ovarian insufficiency (reciprocal translocations of X chromosome with an autosome), undifferentiated gender in 3 (5,3%), dysmorphism and mental retardation in 2 (3.5%). In 5 patients (8.9%) familial rearrangement was diagnosed.
Conclusion: Cytogenetic analysis is an important first step in the overall schedule for genetic investigation that is still invaluable in the diagnostic algorithm of some entities.

J13.09
Loss of the chromosome PAR2 region in a case with a satellited Yq chromosome and attention deficit disorder

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The so-called satellited Y chromosomes (Yqs) are the product of a translocation of the short arm of an acrocentric, preferentially chromosome 15, and the long arm of the Y, and they are considered without clinical consequences. We present a case with attention deficit hyperactivity disorder (ADHD), carrier of a Yqs with a deletion in Yq12. We also speculate about the possible role of genes on sex chromosomes as possible risk factors for ADHD.
We present a 12-year-old boy, second child of a mother who underwent amniocentesis for advanced maternal age. Karyotype in cultured amniocytes and parental blood samples revealed the presence of a de novo Yqs in the fetus. Additional FISH studies showed the chromosome 15 origin of the Yq satellited material. Baby delivery was uneventful. After an apparently normal development, he presented learning difficulties and behavioral problems, compatible with ADHD. Arrays-CGH studies, performed at the age of 12, identified the presence of a maternally inherited 152Kb duplication on 12q24.32 and a 242Kb deletion in the PAR2 region on the X or Y chromosome: arr[hg18]12q24.32(128224063-128376460)x3,Xq28(154600946-15843418)x1,Yq12(57460146-57702618)x1. FISH studies using BAC probes that hybridize with SPRY3 and VAMP7, confirmed the deletion which was localized on chromosome Yq12. The presence of the deletion was ruled out in father by FISH analysis.
Deletion of SPRY3 and VAMP7 genes on Yq12 cannot be considered the cause of the ADHD phenotype, as these genes are genetically inactive in the Y chromosome in males. We cannot rule out, however, loss-of-function genetic or epigenetic defects in the copy of these genes on X chromosome

J13.10
De novo reciprocal translocations are associated with different chromosomes and clinical outcomes

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Balanced chromosomal rearrangements are the most frequent genetic abnormalities in the population (0.3-0.5%) and a major concern in the couples with reproductive problems (2-8%). These problems are usually recurrent miscarriages, chromosomally abnormal offspring or in some cases with infertility. These translocations usually do not show any phenotypic effect in most carriers, but sometimes may cause abnormal phenotype. In this report, the clinical, cytogenetic and molecular cytogenetic evaluations were performed on 13 patients with de novo balanced reciprocal translocation. Eleven of 13 patients have normal phenotype and two cases have dysmorphic features. Seven of 11 patients applied to our department for fertility problems and the remaining 4 have recurrent miscarriages. All these cases were undertaken IVF-PGD programme, by giving the genetic counselling. The other two patients were clinically evaluated for syndromic cases. In these patients, the responsible gene(s) in breakpoint regions were planned to identify for abnormal phenotype.

J13.11
Fanconi anaemia, chromosome instability, DNA replication and fragile sites.

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Fanconi anaemia (FA) is an inherited DNA repair deficiency syndrome caused by biallelic mutations of 1 of the 15 FANC genes. FA cells feature a high frequency of broken and radial chromosomes and are highly sensitive to interstrand DNA crosslinks. Chromosomal aberrations are often associated with incomplete genome duplication following replicative stress. Common fragile sites (CFSs) are usually late replicating regions and it is now common opinion that their expression as chromosome gaps or breaks may be due to unreplicated DNA.
Purpose: To study, within two CFSs, FRA2G and FRA2H, the relationship between DNA replication timing and fragility in FA lymphoblasts mutated in the FANCA gene (FA-A) and in the same cells transfected with normal FANCA gene (FANCA).
Methods: FISH, using sequences mapping within our two CFRAs, on interphase nuclei obtained from FA-A, FANCA and control cells: each locus before replication appears as a single hybridization dot signal (S), while after replication duplicated dot signals (D) are visible. Five distinct temporal S-phase stages can be distinguished from the G1- and G2-nuclei by adding BrdU twenty minutes prior to cell harvest and analysing the BrdU-labelling patterns of the DNA replication.
Results: Respect to control cells, in FA-A and FANCA cells, the fragile sequences show a DNA replication stalling between the second and third stage of S phase. Moreover, while FA-A cells reach the last phase with only a 55% of duplicated loci, FANCA cells show a more regular trend reaching, as the control cells, the last phase of S with about 69% of replicated alleles.

J13.12

Persistence of hidden chromosome instability in peripheral blood lymphocytes of persons occupationally contacted with ionizing radiation

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To research the possible contribution of radiation exposure into persistence of hidden chromosomal instability (HCI) in somatic human cells two groups of observation - occupational group (persons exposed to low doses of ionizing radiation during conversion of Chornobyl's „Shelter" into ecologically safe system) and unexposed matched control group were cytogenetically observed via joint use of two modifying tests - «G2-bleomycin sensitivity assay» and two-termed cultivation of peripheral blood lymphocytes. Under standard short-term (48 hours) cultivation background mean-group frequency of cytogenetic parameters in occupational group did not differ (P>0.01) as from the results of cytogenetic observation of unexposed group as from values typical for spontaneous chromosome mutagenesis in human peripheral blood lymphocytes. Under long-term (100 hours) cultivation mean background cytogenetic effect in occupational group increased (P <0.001) compared with that in first mitosis, whereas in unexposed group it decreased with time. Following testing bleomycin exposure principal differences between the groups in the manifestation and dynamics of cytogenetic effects were revealed. Despite the unidirectionality in dynamics of mean cytogenetic effect in both groups (decline in third mitosis) its mean value in occupational group exceeded (P <0.001) such in unexposed group under both terms of cultivation. Moreover, examined groups differed in interindividual peculiarities of HCI persistence which in occupational group confirmed the possibility not only preservation, but even accumulation of chromosome instability in successive cell's generations. Data received can be used to identify persons hypersensitive to mutagenic exposure of ionizing radiation in view of individual features of HCI expression and persistence.

J13.13

Functional studies of BBS2 gene by minigene assay

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Introduction: Bardet-Biedl syndrome (BBS, #209900) is a genetic rare disease belonging to the group of ciliopathies. Since the molecular basis underlying BBS is not fully understood and the real role of *BBS* variants has to be characterised, we aimed to analyse the functional implications of two *BBS2* mutations (p.Tyr89Cys and p.Arg275*) predicted to alter the normal splicing process after transcription. Remarkably, BBS2 protein is part of the BBSome-core complex, an important intermediate in the assembly of the mature BBSome, which is known to be involved in trafficking to ciliary membrane.

Materials and Methods: We performed mRNA expression studies by minigene assay using *pSPL3* vector and COS-7 cells for *in vitro* experiments. All minigene constructions and cDNA sequences were confirmed by direct sequencing.

Results: The two mutations analysed here were not observed to affect mRNA processing, as wildtype and mutated constructions produced the same pattern after agarose gel electrophoresis. Direct sequencing supported these negative results. Interestingly, we observed that nonsense-mediated decay did not work when p.Arg275* variant was transcribed, since we detected the band corresponding to the mutated transcript.

Conclusions: Although bioinformatic predictions are helpful to focus on molecular studies, biological experiments always have to confirm this data. Despite the negative results obtained here, we cannot discard any kind of alteration in BBS2 protein structure and/or function. Further studies will have to clarify the real role of these variants in DNA processing.

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J13.14

Comparison of human dermal fibroblasts (HDFs) growth rate in culture media supplemented with or without basic fibroblast growth factor (bFGF)

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Basic fibroblast growth factor (bFGF) is a member of the FGF family secreted by different kinds of cells like HDFs and it is an important nutritional factor for cell growth and differentiation. The present study aims to investigate the HDFs growth rate in culture media supplemented either with or without bFGF. HDFs were isolated from human foreskin sample and were cultured in vitro in media containing bFGF and lack of this factor. The karyotyping was performed using G-banding to investigate the chromosomal abnormality of HDFs in both groups. The real-time Q-PCR was used to measure the expression level of p27kip1 and cyclin D1 genes normalized to internal control gene (GAPDH). HDFs cultured in media or without bFGF had normal karyotype and chromosomal abnormalities were not observed. The cell growth rates in both groups were normal with proliferated exponentially but the slope of growth curve in HDFs cultured in media containing bFGF was increased. Karyotyp test showed bFGF does not affect on cytogenetic stability of cells. The survey of p27kip1 and cyclin D1 genes by real-time Q-PCR showed the expression level of these genes were up-regulated when adding bFGF in culture media (p<0.05). This study demonstrates appropriate supplementation of culture media with growth factor like bFGF could enhance the proliferation and differentiation capacity of cells. Similarly, fibroblast growth factors did not induce any chromosomal abnormality in cells. Furthermore, cultured HDFs in bFGF supplemented media, the p27kip1 and cyclin D1 genes were up-regulated and suggesting an important role for bFGF in cell-cycle regulation and progression and fibroblast division stimulation.

J13.15

The frequency of spontaneous and radiation-induced cytogenetic damages in persons aged 12-100 years

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Using G-banding cytogenetics the frequencies of spontaneous and radiation-induced chromosome aberrations in peripheral blood lymphocytes of adolescents, middle-aged, elderly and centenarians were established. The gradual increase of mean-group spontaneous chromosome aberrations frequencies from adolescents to middle-aged and elderly (1.16±0.25, 2.01±0.34, 4.44±0.55 per 100 metaphases, respectively) and decline of spontaneous chromosome mutagenesis intensity in centenarians (2.78±0.55 per 100 metaphases) were found. The scatter of individual values of chromosome aberrations was within the limits of 0.67-1.74, 0.80-3.33, 2.2-6.25 and 1.00-5.76 per 100 cells in adolescents, middle-aged, elderly and centenarians, respectively. Accumulation of translocations and inversions with age in all groups was registered. Under X-radiation in vitro exposure in dose 250 mGy increase of chromosome injuries due to increase of chromosome type aberrations was determined in all groups. There wasn't detected significant difference between the mean-group frequencies of radiation-induced chromosome aberrations in adolescents, middle-aged and centenarians (6.16 ± 0.72, 5.64 ± 0.65, 7.01 ± 0.74 per 100 metaphases, respectively) while in elderly cytogenetic effects appeared higher than in other groups (11.35±0.97 per 100 metaphases). The maximal above-spontaneous level of cytogenetic radiogenic markers was determined in group aged 12-16 which indicates increased radiosensitivity during adolescence. The elevated frequency of chromatid type aberrations was detected in persons aged 60-70 indicating increased chromosome instability. In persons from different age groups chromosomes damaged accordingly to their relative length, especially in euchromatic regions (79.8% and 88.1% under spontaneous and radiation-induced mutagenesis, respectively). The most sensitive chromosome bends to X-irradiation in vitro in each observed age group were determined.

J13.16

Detection of Down syndrome using 6 STR markers

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Introduction: Down syndrome (DS) is a congenital disorder resulting from abnormalities affecting chromosome 21. The goal of this study was to examine the effectiveness of 6 STR markers application (D21S1435, D21S11, D21S1270, D21S1411, D21S226 and IFNAR) in molecular genetics diagnostics of Down syndrome.

Materials and Methods: Testing was performed on 73 children, 45 boys and 28 girls, cytogenetically confirmed to have DS. DNA isolated from the buccal swab was used. STR markers, located on chromosome 21, were simultaneously amplified using quantitative fluorescence PCR (QF PCR).

Results: STR markers D21S1435, D21S11, D21S1270, D21S1411 and IFNAR have shown themselves to be good polymorphism markers in detection of trisomy 21 because they had almost identical number of samples with three

or two alleles (peak ratio of 2:1) on previosly mentioned marker. On the other hand, the D21S226 marker was uninformative for 19 samples as it showed only one allele.
Conclusions: However, the analysis of the other five STR markers have given positive information or the confirmation of trisomy 21.

J13.17
Modification of radiation-induced bystander effect in human peripheral blood lymphocytes in vitro

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Exposure of ionizing radiation accompanied by release from irradiated cells into surrounding medium cytokines and factors that promote the development of secondary oxidative stress and thus induce bystander effect in the unirradiated cells. The aim of the research was study the modification of bystander effect in human peripheral blood lymphocytes exposed to 1 Gy of X-rays via antioxidative vitamin complex (VC) containing water-soluble forms of tocopherol, ascorbic acid and retinol. GTG-banding metaphase chromosomes staining and developed by us "mixed human blood lymphocytes culture assay" consisted of cells differed on cytogenetic sex markers have been used. Mixed cultures received from 6 volunteers of different sexes have been treated by VC in concentration 40 μg/ml before the culturing. The level of chromosome aberrations in non-irradiated bystander cells cocultured with irradiated lymphocytes was 5.34 ± 0.49 per 100 metaphases and exceeded that in control cultures ($p<0.001$). Bystander effect in damaged chromosomes was presented mainly by chromatid breaks (3.97±0.42 per 100 metaphases). The frequency of chromosome types aberrations (deletions, translocations, inversions, dicentric and ring chromosomes) in bystander cells did not differed from control values ($p>0.05$). The modification of bystander effect by VC reduced the chromatid breaks frequency to a level (1.14±0.23 per 100 metaphases) which did not differ from such in control cultures (0.87±0.22 per 100 metaphases) ($p>0.05$). The data received testify about the protection of non-irradiated cells by antioxidative VC from the occurrence of secondary oxidative stress and elimination the development of radiation-induced bystander effect.

J13.18
Effect of spontaneous level of γH2AX foci on the level of cytogenetic damage in human cells

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Introduction: Spontaneous level of DNA damage and radiosensitivity of human cells are characterized by considerable interindividual variability. Mutagen-induced DNA double-strand breaks (DSBs), marked by γH2AX, in cells have traditionally been considered as dangerous pre-mutation damage. However, the nature of spontaneous γH2AX foci is not clear.
Purpose: To analyze the effect of spontaneous level of γH2AX foci on the spontaneous and radiation-induced level of cytogenetic damage in human cells.
Material and methods: Chromosome aberrations, micronuclei and γH2AX foci were assessed in lymphocytes of 54 healthy individuals and 11 patients with solid tumors, and extraembryonic fibroblasts of 18 human embryos.
Results: There was no direct correlation between spontaneous level of γH2AX foci and spontaneous frequency of centromere-negative micronuclei in both lymphocytes and extraembryonic fibroblasts. It suggests that not all spontaneous γH2AX foci, unlike the radiation-induced ones, correspond to DNA DSBs. Furthermore, after irradiation of lymphocytes from healthy individuals a negative correlation was found between spontaneous level of γH2AX foci and frequency of radiation-induced centromere-negative micronuclei (R=-0,37, p=0,025). Checking these results *in vivo* in the lymphocytes of cancer patients demonstrated that level of γH2AX foci before radiation therapy negatively correlated with the frequency of chromosome aberrations (mainly paired fragments) after the end of treatment (R=-0,73, p=0.024), reflecting the individual radiosensitivity.
Conclusion: Although the spontaneous level of γH2AX foci does not reflect the actual number of chromosomal abnormalities, it may be a marker of individual DNA DSBs repair capacity in human cells.
Study was supported by Grant of President №-14.122.13.6806, RFBR grant №14-04-31867.

J13.19
Insulin producing cells from murine Embryonic Stem Cells using Foxo1-siRNA and Gcg-siRNA

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Introduction: Embryonic stem cells (ESCs) are potential pluripotent cells derived from inner cell mass of embryonic blastocyst stage. So far, growth factors have been used for differentiation of ESCs to insulin producing cells . In the present study, in the absence of growth factors, siRNA was used to silence targeted genes.
Materials and Methods: In this study, embryoid bodies (EBs) were derived from murine ESCs. The EBs were then cultured in four groups; three test groups (containing culture medium with siRNA) and the control (the same culture medium used in test group without siRNA). After three weeks, differentiated cells were analyzed by using RT-PCR(expression of some pancreas-specific genes), immunocytochemistry (detection of insulin presence in cells) and ELISA (evaluation of the amount of secreted insulin to culture medium).
Results: The RT-PCR analysis of differentiated cells on three test groups showed expression of beta cell specific markers including insulin and Pdx1. The results of immunostaining showed that the insulin protein are expressed in differentiated cell of Foxo1 siRNA group and Foxo1/Gcg-siRNA group with different amounts and finally insulin secretion assay show that differentiated cells on Foxo1 siRNA group secreted more insulin in comparison with the other groups.
Discussion: Our data indicate that murine ESCs differentiate into insulin producing cell using siRNA ,without growth factors. Therefore, siRNA can be used as a novel approach for generating insulin producing cells from ESCs in vitro.

J13.20
The profile analysis of 500 genes expression associated with immune system's function at the mentally retarded patient irradiated in utero due to the Chernobyl accident

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The main types of medico-biological consequences of radiation on human genome is genomic instability leading to malignant growths, intellectual backwardness, hereditary (chromosomal/gene) diseases in children from irradiated parents. The analysis of hospitalized diseases incidence showed a considerable share of intellectual retardation (IRD) in children irradiated in utero after the accident on the Chernobyl NPP. Data of IRD association with dismorfogenesis of brain and cerebral vessels is obtained (MRI-investigations).
The profile analysis of 500 genes expression associate with immune system's function which is carried out using the analyzer nCounter (Nanostring technologies, USA) showed statistically significant distinction ($p< 0.05$) to 10 genes expression (the raised expression - for genes IL1B, CXCL1, PTGS2, EGR2 and lowered - for IL17B, KIT, BATF3, CD83, CCL16, ATG12) at the mentally retarded patient irradiated in utero.
Children from a group of comparison have different immunopathological states. The further Bioinformatic analysis revealed changes of of genes expression functioning: IL1B, CCL16 participating in inflammatory reactions in nervous system, genes of EGR2, BATF3 - in regulation of a transcription of genes, genes of KIT, ATG12 - in regulation of process of apoptosis, IL17B gene - with nervous system functioning.
Changes in an expression of genes CXCL1, PTGS2, CD83 have association with oncogenic processes (according to scientific literature).
Our original research data concerning a characteristic of 10 genes profile in immune system's function will make a contribution in understanding of mechanisms of intellectual backwardness formation as a result of radiation pre-natal exposure and the radiation induced cancerogenesis.

J13.21
THE STUDY OF SODIUM LIGNOGUMATE AS MODIFICATOR OF RADIATION-INDUCED MUTAGENESIS

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The objective of the study - complex cytogenetic analysis of sodium lignohumate (SLH) exposure under mutagenesis induced by γ-irradiation in Allium-test. Analysis of root meristem cells of Allium cepa L. seeds by usage of ana-telophase method had were carried out. The effects of SLH in concen-

tration of 100 mg/l on cytogenetic effects induced by γ-irradiation (Cs137) in doses of 5, 10 and 20 Gy was investigated. Polyfunctionality of SLH as potential antimutagen is ensured by the presence of not only antioxidant properties, but also a number of other mechanisms. Under SLH impact more effectively repair of chromosome damages in cells with one aberration took place, while in cells with a large number of chromosome injuries less efficient repair processes were registered. Stimulation of repair processes induced by SLH depended on the radiation dose. It was most effective under the dose of 5 Gy. With increasing of radiation dose up to 10 and 20 Gy this process deminished because it included other anti-mutagenic mechanisms: stimulation of repopulation and apoptosis, which are prevalent in the implementation of the final gene-protective effect. Differential antimutagenic activity of SLH in relation to various types of aberrations was revealed: the most effective reduction the frequency of short-lived markers of radiation mutagenesis (chromosome type aberrations) was detected; lower efficiency for long-term potential changes in the chromosomes, which then realized in chromatid type aberrations was showed. Thus, multiple mechanisms for implementing antimutagenic properties of SLH in γ-irradiation-induced mutagenesis in Allium-test were revealed. The data received permit to suppose that SLH can be use as therapeutic radioprotector.

J13.22
Post-replicative chromatin's role in formation of chromosomes and sex chromatin

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Distinctions between a pre-replicative and post-replicative chromatin remained imperceptible so far and didn't draw attention of researchers. However they were important for an explanation of fundamental structurally functional properties of a chromatin.
Replication of a threadlike chromatin doubles quantity of DNA in S period of an interphase. However, the quantity of the histone disks remains without change. Therefore, replication forms a new post-replicative form of a chromatin with double deficiency of histones (semihistonal chromatin or semichromatin). Spiralization and condensation are the main properties of a semichromatin. Semichromatin almost completely retained deficiency of histones and exposed to spiralization from interphase period G2 to a prometaphase, i.e. before merging of a nucleoplasm to cytoplasm. Free histones of cytoplasm compensate for the deficiency of histones of a semichromatin of chromosomes during an anaphase and a telophase. In such a way semichromatin of chromosomes turns in full on histones threadlike chromatin with a diameter of 11 nanometers.
All interphase nuclei contain chromatin bodies and nucleoreticulum. Nuclei of female cells have still sex chromatin. All of them represent the remains of a semichromatin of chromosomes which didn't manage to fill completely the missing histones and kept a spiralized form of a post-replicative chromatin. Preservation of the semichromatin parts of chromosomes in G1 period of an interphase can appear one of ways of long blocking of activity of seldom included genes. It is possible to explain with this property of a post-replicative chromatin the mechanism of blockade of genes of X chromosome in a sex chromatin.

J13.23
Genotype-endophenotype correlation in patients with neurodevelopmental disorders and chromosomal microrearragements.

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Introduction: Rare CNV are frequently detected in patients with neurodevelopmental disorders, however, the relationship between pathogenic CNV and/or impaired dimensional neuropsychological traits need to be defined.
Material and Methods: We assessed a systematic analysis of the neuropsychological phenotype of 410 patients (56% M), age-range 24 months-18 years, affected by ID, ASDs, ADHD, communication disorders, tics and related-disorders attempted a correlation with CGH genotype.
Results: In 91 out of 410 patients (24.8%) 98 microrearrangements were detected by array-CGH among which 8 were disease-causing and 90 variants of uncertain significance, potentially pathogenic. Groups of patients with overlapping microrearrangements were identified and their neuropsychological profiles were systematically analysed (language, memory, attention, executive functions, visuo-spatial skills). Patients with chromosomal rearrangements showed more frequent facial and/or somatic dysmorphisms and major rate of comorbidities between ID with the other neurodevelopmental disorders. Chromosomal rearrangements associated with overlapping CGH included: 15q11.2 deletion comprising TUBGCP5, CYFIP1 and NIPA1-NIPA2 genes involved in neurogenesis, 15q11.2 duplication encompassing the autism susceptibility genes GABRA5 and GABRB3, 22q11.2 deletion and duplication affecting PRODH/DGCR6, TBX1, COMT and ZDHHC8 genes involved in neurotransmission and neurotoxicity, a gain-of-function Xp22.32 encompassing VCX3A and STS genes reported in patients with X-linked ichthyosis and ID.
Conclusions: Patients with somatic signs and lower cognitive levels associated to other neurodevelopmental disorders might be more likely to present CNV. The study of genotype-endophenotype relationship may support clinical diagnosis, prognosis and personalized rehabilitation programs.

J13.24
Mixoploidiy: A surviving case of 10-years-old with diploid-tetraploid with no mental retardation

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Mixoploidy is the presence of a normal diploid cell population with 3 or more multiples of the haploid chromosome number. In 2n/4n mixoploidy the chromosome complement of the abnormal line is 92. The aim of the present study is to describe a case with mixoploidy. The proband, a 10-years-old male, was the second product of healthy, young and non-consanguineous parents with normal pregnancy. At birth, he weighed 1200 g and heighted 43 cm. Currently on physical examination, he showed a height of 103 cm (>3th percentile), weight of 17 kg (>3th percentile), head circumference of 48 cm (>3th percentile), normal IQ, pitched voice, microcephaly, brachycephaly, upper hairline anteriorly and posteriorly, ocular protrusion, downslanting palpebral fissures, hypertelorism, telecanthus, broad nasal bridge, dysplastic ear, low set ear, large philtrum, thick lips, wide mouth, dental malocclusion, hypoplastic mandible, small hands with brachydactyly and tips pointed, camptodactyly, coxa and geno varus, and pelvic limb shortening. At the age of 3-years-old, kariotype result in blood was mos 92, XXYY[35]/ 46,XY[65] and in fibroblasts mos 92, XXYY[42]/ 46,XY[58]. Currently, his kariotype in blood was mos 92, XXYY[73]/ 46,XY[27] and in fibroblasts mos 92, XXYY[71]/ 46,XY[29]; FISH analysis revealed nuc ish (DXZ1x2,DYZ3x2,D18Z1x4) [164]/ nuc ish (DXZ1x1,DYZ3x1,D18Z1x2)[39]. Genetic profile using 16 DNA markers showed only the presence of diploid complement. 2n4n mixoploidy seems to be due to a failure of cytokinesis after the first mitotic division of the zygote; other possibility is that a 2n zygote and a 4n zygote generate a chimera; in our case the former mechanism could be the cause of this mosaicism.

J13.25
An unusual association of a deletion on Xq21 in a Mosaic Turner female with mental retardation, seizures, facial and body asymmetry

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Turner's syndrome affects about one in 2000 liveborn females. In about 50% of cases, karyotype analysis reveals the complete loss of one X chromosome, whereas the remaining patients display a multitude of chromosomal X abnormalities. Most people with Turner's syndrome have normal intelligence, however mental retardation was observed in patients with small ring X chromosome and terminal X deletions because of deletion of XIST or absence of skewed X inactivation, respectively.
We report the case of a twelve-year-old girl who was referred to our genetic clinic because of dysmorphic features, mental retardation and epilepsy. Craniofacial abnormalities included facial asymmetry, broad forehead, hypertelorism, bulbous nose, puffy cheeks and lower lip thick. The height was 104 cm (-4DS), and she had atrophy of the right hemicorpus, torso achromic lesions bands and absence of mammary development. Conventional cytogenetic analysis using G-bands revealed a 45,X/46,X,del(X)(q21.1) karyotype. Parental karyotypes were normal. Array-CGH refined the breakpoint location on Xq21.1, showed the absence of deletion of XIST and revealed the absence of other pathogenic copy number variations (CNVs). X-inactivation analysis with cytogenetic method will be considered.
Our observation is unique because facial and body asymmetry were never reported with mosaic Turner females. Usually large deletions are likely to be associated with complete skewed X inactivation when XIST is present so that the resulting phenotypes are relatively benign given the amount of

genetic material missing. The phenotype of our patient might be explained by inactivation of the normal X chromosome in cells with a deleted X.

J13.26
Digenic inheritance in Mexican patients with hereditary sensorineural hearing loss revealed through exome analysis

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Background: Hereditary neurosensorial hearing loss (NSHL) is a genetically heterogeneous disorder worldwide. GJB2 gene affections are a frequent cause of hereditary NSHL with a wide spectrum of clinical data that ranges from moderate to profound NSHL. Mexican population harbours a low frequency of GJB2 homozygous mutations in NSHL. In some cases, digenic inheritance is a cause of NSHL Objective: To describe two novel digenic mutations in the GJB2 gene and OTOA and MYO1C genes in patients with NSHL in a sample of Mexican patients. Materials and methods: Two families with prelingual NSHL were included in the study. Genomic DNA was extracted by conventional methods for exome analysis in affected patients. DNA sequencing analysis was performed to confirm exome findings. Results The digenic inheritance corresponded to GJB2 c.35delG in both families and OTOA p.E787X and MYO1C p.E831K in each family, respectively; both genes associated to NSHL. Parents were tested for these gene mutations and they had the heterozygous state that allowed to confirm the recessive inheritance pattern. These mutations were searching in 100 normal controls to discard a possible polymorphism. Conclusion: We describe two novel digenic varieties of homozygous mutations in patients with NSHL. All patients presented profound hypoacusia with no other anomalies. These data show the complexity in the genesis of NSHL and that the genetic defects are greater than expected in Mexican population.

J13.27
Modification of plasmid antisense strand for in vivo transfection.

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Plasmid vectors are more preferable then viral ones since the former do not cause immune reaction, have relatively small size, and can be easily produced. Plasmid vectors do not integrate in chromosomes and are more stable. They might be used for treatment of monogenetic diseases, attribution new functions to cells, and various strategies for cancer therapy. However, nowadays plasmid vectors are not effective enough due to absence of stable bond with cell-penetrating peptide (CPP). The earlier solutions of the present problem were not successful enough. Non-covalently modified vectors are too large and chemical modifications affect the target genes. We suggest plasmid modification with biotin. By means of biotin-streptavidin interaction this plasmid might be strongly bound with CPP.
Prokaryote's transcription occurs only on the anti-sense strand of DNA and doesn't use the sense one. It allows the modification of the sense strand of plasmid to provide stable construction for gene therapy. Two types of plasmids were designed which contain green fluorescent protein gene downstream the eukaryotic promoter. In the first plasmid a part of thymine bases was replaced by biotinylated uracils on the sense strand of DNA. Similarly, in the second plasmid the anti-sense strand of DNA was modified.
The analysis of the biotinylated plasmid restriction fragments by immunoblotting confirms that both plasmids are biotinylated along the whole length. Peroxidase-labeled streptavidin was used for detection. The ability of the plasmids obtained for expression of green fluorescent protein was tested on the HEK 293 cell line. Expression of the target gene was observed only in the case of the sense strand modification.

J13.28
Impaired DNA damage response to oxidative stress in Rubinstein-Taybi syndrome cells

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Rubinstein-Taybi syndrome (RSTS OMIM #180849, #613684), is a rare genetic disorder characterized by mental impairment, skeletal abnormalities, growth deficiency, and an increased risk of neoplasia. About 60% of RSTS patients carry a heterozygous mutation in CREBBP or EP300 genes encoding the homologous CBP and p300 acetyltransferase proteins that play a key role in DNA repair.
In order to investigate a possible altered DNA damage response (DDR) concurring to RSTS pathogenesis we analyzed lymphoblastoid cell lines (LCLs) derived from 5 patients carrying CREBBP (2 missense in KAT domain and a whole gene deletion) or EP300 (2 precocious frameshift) mutations to assess their ability to respond to different types of DNA damage.
All the analyzed RSTS cell lines showed i) signs of the presence of endogenous DNA Damage, as assessed by the appearance of phosphorylated histone H2AX, ii) increased sensitivity to oxidative potassium bromate-induced DNA damage and iii) significantly reduced ability to perform DNA incision after DNA damage, i.e. the first step of the base excision repair (BER) process carried out by a DNA glycosylase. The acetylation of DNA polymerase beta, DNA glycosylase OGG1, PCNA, histone H3 and also auto-acetylation of CBP and p300 are all reduced in RSTS cells, suggesting a correlation with BER deficiency.
All these findings support our rationale on impaired DDR, particularly at the level of BER, in RSTS cells, thus suggesting that full CBP/p300 activity is required for successful DNA damage removal and for maintainance of cell functions homeostasis.
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J13.29
Beare-Stevenson and Crouzon syndromes: spectrum of the same disease. Identification of a novel mutation in the FGFR2 gene

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Beare-Stevenson syndrome (BSS,MIM 123709) is a syndrome characterized by craniofacial anomalies, craniosynostosis, ear defects, cutis gyrata, acanthosis nigricans, anogenital anomalies, skin tags, and prominent umbilical stump. Crouzonoid-like features are present in some cases whereas a few patients have a broad range of phenotypic features. Currently, a dozen of patients with FGFR2 gene mutations and BBS have been described; mutations p.Y375C and p.S372C. are the most characteristic point mutations in the FGFR2 gen and are present in 50-60% of patients with BBS. As not all patients have FGFR2 mutations, genetic heterogeneity has been proposed. The proband, a 21-years-old female, was the second product of healthy, young and non-consanguineous parents with normal pregnancy. At birth, she weighed 1500 g and heighted 39 cm. Currently on physical examination, she showed a height of 139 cm, weight of 41 kg, head circumference of 51 cm, borderline IQ, brachycephaly, sagittal suture and left coronal suture prominent, redundant skin in occipital region, low hairline posteriorly, ocular protrusion, upslanting palpebral fissures, hypertelorism, telecanthus, broad nasal bridge, broad and bulbous nose, short philtrum, thick lips, lip corners down, wide mouth, dental malocclusion, hypoplastic mandible, left geno valgus and left pelvic limb shortening. Genomic DNA analysis showed a novel p.F409I mutation within exon 9 of the FGFR2 gene. This mutation was not found in 100 normal controls or normal parents excluding a polymorphism. We describe a patient with mild clinical characteristics of Beare-Stevenson and Crouzon syndromes which enriches the spectrum of mutations in the FGFR gene and remarks the clinical variability overlapping of the symptoms of both diseases.

J14.01
Application of clinical exome sequencing in diagnostic setting

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High throughput sequencing has made possible the analysis of the human whole exome in clinical setting, raising technical, bioinformatics and interpretation challenges. We analyzed by clinical exome sequencing (CES) 130 patient-parents trios aged under 14 years, affected by cardiovascular, liver or neurologic disorders with suspected genetic causes. Our aim was the substitution of traditional gene-centered approach with a comprehensive analysis of all known disease genes.
Of the 130 patients, 61 (47%) carried at least one potentially causative mutation. Among them, 42 had an autosomal dominant disease of which 24 (57%) occurred de novo, 17 had autosomal recessive disease and two had X-linked disease. CES confirmed the clinical suspicion in 68% of cases, in 15% completely changed the initial clinical diagnosis and in 17% suggested a possible diagnosis.
Although the high detection rate observed, 53% of patients remained undia-

gnosed, of which 15% had positive family history and/or clinical presentations that strongly suggested a genetic basis.
We conclude that CES allows the rapid diagnosis of rare and heterogeneous genetic conditions in a unique and standardized laboratory workflow, but this type of analysis cannot be considered definitively negative because of the rapid improvement of genetic knowledge. Thus we suggest that a clinically optimized whole exome sequencing should be considered for clinical diagnosis of heterogeneous genetic conditions, focusing the analysis on known disease genes related to the patient's phenotype. By this approach, the report should be considered as a dynamic process, periodically revised on the basis of new clinical evidences or new research discoveries.

J14.02
Array Comparative Genomic Hybridization (array-CGH) for the genetic diagnosis of congenital heart defects

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Congenital heart defects (CHD) are the most common developmental anomalies and the leading non-infectious causes of mortality in newborns. The Microarray-based comparative genomic hybridization (Array -CGH) technique proved useful for the detection of submicroscopic chromosome rearrangements that could not be detected by conventional cytogenetic methods. **Objective**: The project aimed at the identification of inherited or/ novel genome variants causing syndromic or non- syndromic heart defects. **Subjects & Methods**: A total of 83 patients with CHD associated with developmental delay or other malformations were recruited in the study. We applied conventional cytogenetic methods such as G-banding and Fluorescent In-Situ Hybridization (FISH) techniques concomitant with high density array-CGH using 2X 400 oligo- microarray (Agilent). We could detect chromosome abnormalities in 11/83 (13%) cases using conventional cytogenetic techniques: Trisomy 18 (2), trisomy 13 (1), ring 18 (1), trisomy 9p (1), (del22q11.2) (3), (del7q11.23) (2), del 18(p11.32-p11.21) (1). Application of array-CGH technique confirmed the previous results and revealed pathogenic variants in another 10/33 samples (30%), besides large number of copy number variants (CNVs) were observed in all studied samples. **Conclusion**: the diagnostic yield of array-CGH technique is much higher than that observed using conventional cytogenetic techniques and proved to be useful for the clinical diagnosis of affected patients. The analysis of gene content in CNVs may be useful for detection of molecular pathways of cardiac development.
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J14.03
Evaluating the economic costs of clinical exome sequencing in a clinical genetics service laboratory

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In 2014, the regional clinical genetics laboratory at Cambridge University Hospitals, UK, introduced targeted clinical exome sequencing as a routine molecular diagnostic service. This offers potentially the best attributes of both the targeted gene panel and whole exome sequencing approaches. Initial tests were run on an Illumina HiSeq2500 but we then transferred the tests to a NextSeq500.
Here we report an economic analysis of this approach, detailing a cost consequences analysis from the perspective of the molecular genetics laboratory of prospectively testing approximately 900 patients chronologically referred for diagnostic testing over a period of 12 months. We compare: (a) costs associated with running the test on two different platforms, the Illumina HiSeq2500 and NextSeq500; and (b) differences in cost between this clinical exome sequencing approach and alternative conventional approaches which are available entailing Sanger sequencing and/or laboratory based NGS gene panel tests. The costs covered the diagnostic testing pathway and included laboratory work, machine costs, reagent costs, bioinformatics, confirmatory testing and result interpretation plus overheads as appropriate. Initial findings for the costs associated with a clinical exome sequencing approach suggest a potential average saving of between 15-25% per patient tested, compared with the cost of alternative available genetic tests.

J14.04
Bisulfite Sequencing Polymerase Chain Reaction for Analysis of the Promyelocytic Leukemia (PML) Promoter

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DNA methylation in vertebrates typically occurs at cytosine-phosphate-guanine sites (CpG sites). In these regions DNA methylation inhibits binding of active transcription factors and gene expression. In this study we have used two online software programs, MethPrimer and MSPPrimer, to conduct a search of the promyelocytic leukemia (PML) promoter, as a tumor suppressor protein, for the presence of CpG sites or CpG islands. MSPPrimer software predicted a large region to be the CpG island; MethPrimer software predicted a smaller region as the CpG island which was located inside the former. For bisulfite sequencing polymerase chain reaction (BSP) both softwares suggested primer pairs for a common region of the predicted CpG island. Bisulfite-treated DNA from NT2 cells was successfully amplified by the proposed primers. At BSP, in addition to the PCR buffer, we used an ammonium sulfate buffer (AMS) and general Taq polymerase to amplify bisulfite-treated DNA. Together, the results of this study showed that both softwares worked in parallel. Bisulfite-treated DNA was easily amplified with the AMS buffer and general Taq polymers.

J14.05
The application of molecular genetic methods for diagnostic of idiopathic epilepsy in children

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Molecular genetic testing of SCN1A gene is mainly used for diagnosis of SCN1A-associated epilepsy. Mutations in SCN1A gene are the most common cause of generalized epilepsy with febrile seizures plus (GEFS+), severe myoclonic epilepsy of infancy (SMEI; Dravet syndrome) and rare cases of familial hemiplegic migraine. Selection of patients for genetic testing was carried out together with epileptologists among patients with idiopathic epilepsy (IE) observed in Medical Center. The five pathogenic mutations in SCN1A were determined in 45 patients: c.3022G>T, c.1144G>T, c.80G>C, c.3604C>T, c.1131A>C. The material from both parents was available for study in case of mutations detection c.80G>C and c.3604C>T. In the first family, mutation c.80G>C was detected in the mother without clinical features of epilepsy. There were no similar changes of SCN1A gene in the parents of patient with c.3604C>T. Unfortunately, genetic nature of GEFS+ and SMEI can not be confirmed only by sequencing SCN1A gene in many cases. As part of the research work, we developed panel, consisting of 34 genes, to examine of patients with IE. Total 90 patients with epilepsy were examined. The following number of mutations were found: SCN1A-7, SCN2A-6, SCN1B-3, SCN9A-4. Mutations in SCN1A, SCN1B, SCN2A, SCN9A genes were simultaneously detected in 2 patients. Clinically significant mutations in GRIN2A gene were determined in 2 children; mutations associated with Aicardi-Goutieres syndrome (RNASEH2A, RNASEH2B, TREX1) - in 7; mutation in SLC25A22 gene associated with early epileptic encephalopathy - in 3. There were no mutations in only 7 of all observed children, but 2 patients had deletion of SCN1A gene locus determined by chromosomal microarray analysis.

J14.06
The Investigation of pH Pump Based on Sodium-Bicarbonate Treatment for Acidosis Caused Disease

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The blood pH should be maintained in very narrow limits for intracellular enzyme activities performed adequately and to protect the integrity of the cell membrane. Life-threatening blood pH is considered as the limits between 6.80 and 7.70. When the pH level falls below this value (pH <7.35) acidosis is occured. Acidosis, which occurs in the blood because of the excess of acidic materials is a severe illness. Acidosis could create mortality risks (eg, cardiovascular damage, kidney stones, hormonal problems, osteoporosis, etc.) for patients, unless the mortal risk factors could be eliminated by balancing biologically. To ensure this balance, bicarbonate (HCO_3), protein buffers of methionine and cystine, electrolite buffers of sodium and potassium materials are defined in the medical literature. Sodium bicarbonate is the body's most important buffer system. Due to this, bicarbonate has high concentration in the body and the hemoglobin is under the effect of this buffer. It is the most widely used in clinics and most effective acidosis stabilizer. In this study, the pH pump receptors will located in the abdominal area and

will perform measurements with chemical sensors. The amount of needed pH will be calculated by the software that integrated in the pH pump device. And the necessary sodium-bikarbonate will be pumped to the body, thus the pH level will be stabilized again and again. This project will be a precursor of medical literature and will lead to other studies. Also, In this project, we aim to increase the quality of life by producing the pH pump in order to diagnosed individuals against acidosis caused diseases.

J14.07
Application of a workflow classification of BRCA1 and BRCA2 sequence variants

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Introduction. The likelihood of an unclassified variant result in BRCA1/2 is even higher for individuals from understudied populations.The IARC group has a clinical translation in which is provided a classification system in 5 levels. Not all variants are classified by this system,so we propose design a classification of all variants detected in our laboratory exploiting the information from the IARC system and literature.
Material and methods. Sequence variants classification criteria detected among 662 high-risk breast cancer families;Class 5(pathogenic):nonsense/frameshift variants or IARC level 5[1];Class 4(likely pathogenic):IARC level 4[1] or level 3S[2](splicing variants);Class 3(uncertain clinical effect):IARC level 3[1] or level 2S[2](splicing variants);Class 2(likely not pathogenic):IARC level 2[1] or level 1S[2](splicing variants) or variants described in Deffenbaugh[3] or Tavtigian[4] or using the recommended cut-off of 100:1 in favour of neutrality[5;6] or described in trans with a deleterious mutation and occur at a frequency≥1% in our population;Class 1(not pathogenic):IARC level 1[1],variants reported in control groups at an allele frequency≥1%.
Results. We classified 213 distinct variants:45 class 5 variants,1 class 4 variant, 59 class 3 variants,26 class 2 variants and 111 class 1 variants. Among families:106 families were class 5 variant carriers(16%),2 families were class 4 carriers(0.3%),59 families were class 3 carriers(8.9%) and 100% of families were class 2 or class 1 carriers.
Conclusions. A classification system for genetic variants with recommendations for action coupled to each class would help facilitate the communication between clinicians and laboratory.
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J15.01
A case report of suicide in a teenager with long-term use of fluoxetine associated with CYP2D6 polymorphism

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Background: CYP2D6 enzyme is responsible for the metabolism of antidepressants, including selective serotonin reuptake inhibitors, such as fluoxetine. Genetic variations in the highly polymorphic CYP2D6 gene may be associated with increased, reduced, or lost enzyme's activity, which can cause adverse drug reactions. Researchers discovered a link between fluoxetine use and suicidal behavior among teenagers.
Case presentation: A 17-years old teenager, a girl, has been taking fluoxetine for three months following neurologist's advice as a treatment for mood instability with a prevalence of depressed mood. The teenager committed suicide. After analyzing the girl's diary, the psychiatrist made a conclusion that the girl had been suffering from schizotypal personality disorder. Genotyping showed the presence of heterozygous CYP2D6*4 allele (c.1846G>A) and heterozygous CYP2D6*10 allele (c.100C>T), which may determine decrease in CYP2D6 activity. No other genetic variants (CYP2D6*3, CYP2D6*6, CYP2D6*9, CYP2D6*41) were found. Forensic chemistry research of bodily fluids pointed to presence of fluoxetine in blood and urine. Quantitative measurement of fluoxetine concentration in blood wasn't made because of insufficient volume of blood.
Conclusions: Fluoxetine use may cause toxic drug accumulation thus leading to worsening of suicidal thoughts if taken by teenagers with possible genetic determined insufficiency of CYP2D6 enzyme (poor metabolizer).

J15.02
A tale of genetic variation in the human SLC22A1 gene encoding OCT1 among type 2 diabetes mellitus population groups of West Bengal, India

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The organic cation transporter 1, OCT 1 (also called SLC22A1-Solute Carrier Family 22 member 1), appears to play a role in the efficacy and disposition of variety of organic cation including drugs. Genetic polymorphisms in the drug
transporter have been increasingly recognized as a possible source of variation in drug disposition and response. Genetic variants in OCT1 have been identified largely in European, Asian (Japanese, Chinese and Korean) populations.
Interestingly, eight genetic variations were found in the human SLC22A1 gene, which encodes OCT 1, from 50 type 2 diabetes mellitus individuals (T2DM), in West Bengal population. The purpose of this study was to investigate genetic
variants of OCT1 in West Bengal populations. We detected the three previously reported non-synonymous variations, 480 G>C (L160F); 1022 C>T (P341L); 1222 A>G (M408V) and one synonymous variations 156 T>C (S52S) at a minor
allele frequencies (MAF) of 0.63, 0.20, 0.43 and 0.27 respectively. We also found four previously reported intronic variations: IVS1-43(T>G), IVS2 -99(C>T), IVS5 -61(G>A), IVS9 +43(C>T) with minor allele frequencies of 0.20, 0.17, 0.18, and 0.37 respectively.

J15.03
Scientific justification the tactics of individual pharmacological correction in patients with Duchenne/Becker muscular dystrophy

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Have been justified the hypothesis of modifying influence of the genes involved in methionine and folate cycle (MFC) as well as eNOS gene on the progression of myopathic process.
Materials and Methods: The study was carried out on the territory of Moldova, covering population living in all 39 districts of the Republic. The study period was 24 years, from 1991 to 2014. Epidemiological studies have been conducted: a retrospective cohort study and case-control study. For statistical analysis were used SPSS program and the MDR program.
Results: The prevalence of hereditary neuromuscular pathologies in RM was 23,5:100000 population. Have been identified 17 novel mutations in DMD patients (not described in LMD pages). Evaluation of genotypes association and risk of worsening of myopathy process has identified a statistically significant value for G66G allele of MTRRgene (OR=7.20,p=0.039) and heterozygous carrier status of A2756G mutations in MTRgene (OR=0.63,p=0.045) and carrier status of 4b allele of eNOS gene (OR=1.58,p=0.027). Polymorphic variants of genes involved in MFC and eNOS genes contribute to predisposition to the progression of myopathy process at the molecular level that can explain the nature of clinical polymorphism in development of disability. The genetic basis of susceptibility to progression of the myopathy process and early disability is evident synergy between FMC and eNOS genes.
Conclusions: FMC and eNOS genes are prognostically valuable markers in forecast of the age of stage of wheelchair dependency of DMD/B that substantiates the need for use in clinical practice of neurologists and geneticists for determining the groups at risk of progression of myopathy and personalized therapy of patients

J15.04
An intronic variant of organic cation transporter 1 (Oct1) is associated with all cause and cardiovascular mortality in metformin users.

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Introduction:
Metformin is the most commonly prescribed drug in type 2 diabetes and showed to be associated with decreased risk of incidence of various types of cancer. Metformin is a substrate of organic cation transporters (Octs), especially Oct1, Oct3 (liver) and Oct2 (kidney), which determines the pharmacokinetics/dynamics of metformin and thus its action. There is a great variability in the clinical response to metformin e.g. glycaemic control, mainly due to genetic variances.
Methods:
Genotypes of intronic SNPs in Oct1, Oct2 and Oct3 were determined in the LURIC study a prospective cohort of 3316 male and female participants [mean age 62.6 (10.6) years] scheduled for coronary angiography between 1997 and 2000. We investigated whether intronic variants of Oct1, Oct2 and Oct3 were associated with all cause death and cardiovascular death in metformin users.
Results:
In a multivariate Cox regression analysis adjusted for classical cardiovascular risk factors, the intronic Oct1 SNP rs461473 genotypes were significantly associated with all-cause mortality (p=0.009) and cardiovascular mortality (p=0.0028). An additive Cox regression model showed a decrease in hazard ratio (HR) of 9.57 (95% confidence interval 1.75-52.42) for all cause death as well as of 20.81 (CI: 1.38-313.99) for cardiovascular death, respectively, for each present G allele.
Conclusions:
An intronic variant in Oct1 is associated with all cause and cardiovascular death in metformin users.

J15.05
CYP 2C9 polymorphisms distribution in anticoagulant treatment candidates

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Individual variability in drug efficacy and drug safety is a major challenge in current clinical practice, drug development, and drug regulation. I.e. warfarin or coumarin is a typical drug example of how functional variants of genes affect drug metabolism which is shown in relation to Cytochrome P4502C9 polymorphisms. Cytochrome P4502C9 (CYP2C9) is largely responsible for terminating the anticoagulant effect of warfarin via hydroxylation of the pharmacologically more potent S-enantiomer to inactive metabolites. Mutations in the CYP2C9 gene result in the expression of three variants: CYP2C9*1, CYP2C9*2 and CYP2C9*3.
Main aim of this study was to evaluate the distribution of CYP2C9*1, CYP2C9*2 and CYP2C9*3 polymorphisms within patients who are candidates for anticoagulant (warfarin) treatment. Consequently, the potential side effects of the treatment could be evaluated.
We studied the random distribution of CYP2C9 polymorphisms in patients-candidates for anticoagulant treatment: first patient group was consisted of patients that had at least one thrombo-embolitic episode in their lifetime; second patient group was consisted of women that had recurrent spontaneous abortions (at least two). A control group gathered healthy volunteers who did not have any thrombo-embolitic episode or family history of thromboembolism. Cytochrome 2C9 polymorphisms were genotyped using ARMS PCR method.
Statistically significant over-representation of CYP2C9*2 genotypes were observed. Positive allelic CYP2C9*2(Cys144Arg) and genotype overrepresentation for CYP2C9*/CYP2C9*2 (P=0,0010) within secondary infertility-group. No statistically significant association for CYP2C9*3 variant with either investigated disorder was observed (p>0,05). Observation may imply that significant reductions in intrinsic clearance of a variety of 2C9 substrates could be a contributing risk-factor.

J15.06
Genetic polymorphism of enzymes in patients suffering from acetaminophen overdose

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Introduction: Acetaminophen (APAP) is a mild, analgesic drug used extensively all over the world. APAP has only few side effects if used at therapeutic doses. However, APAP has a narrow therapeutic index. Even a minor overdose of APAP is associated with significant morbidity and mortality and is a major cause of acute liver failure in most western countries.
Genetic variability may predispose some individuals to be at higher risk of being APAP poisoned.
The aim of this study was to investigate if genetic polymorphisms in genes relevant for the metabolism of APAP are associated with the clinical outcome of an APAP overdose.
Methods: A total of 102 patients admitted after APAP overdoses were enrolled in the study. A total of 94 patients were considered to have intentional and eight patients unintentional APAP overdoses. Thirteen patients died or developed hepatic encephalopathy in relation to APAP overdose.
All patients and 50 healthy Danish controls were genotyped using the Sequenom iPLEX® ADME PGx Panel designed for the Sequenom MassARRAY®.
Results: The analysis of the patients showed that the mean dose of ingested APAP was 35.8 g (±29), and the mean ALT level was 5,333 IU/L (±4,775 IU/L). Analyses showed that patients heterozygous for the GSTP1 rs1138272 were overrepresented among patients who either died or developed hepatic encephalopathy/renal failure, with an odds ratio of 3.32 (95% CI. 1.09-10.22, p = 0.034).
Conclusion: The results suggest that rs1138272 may be associated with the clinical outcome of an APAP overdose.

J15.07
Machine learning algorithms based on genotype data predict subgroup of refractory Crohn's disease patients requiring biological therapy

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Crohn's disease (CD) is in addition to ulcerative colitis (UC) one of the two main subtypes of inflammatory bowel disease (IBD). Refractory CD patients, who are not responding to standard therapy and/or are showing side effects during therapy, develop severe disease and fulfill criteria to be enrolled into treatment with TNF-alpha antagonists (infliximab and adalimumab). Aim of this study was to show that refractory CD is a sub-phenotype of CD patients with significantly different genetic architecture and to develop genotype profiles that could most efficiently identify and predict the refractory CD patients. Genotypes for 8.858 LD-pruned single nucleotide polymorphisms (SNPs) of 179 CD patients, including 92 refractory patients were obtained from a custom array Immunochip. Support Vector Machine learning algorithms were used for genotype profile modeling.
We have found that most efficient genotype profile distinguished refractory patients from other CD patients with the best-achieved AUC 0.64 and consisted of 59 discriminator SNPs. Inclusion of demographic data (smoking, age, sex) did not significantly improve the predictive model. In addition, discriminator SNPs were tested in a standard association test between CD and CD refractory patients and two SNPs, rs9592040 in DIAPH3/TDRD3 gene region and rs346818 in KIF1C/GPR172B gene region remained significantly associated after Bonferroni correction.Connectivity analysis (GRAIL) revealed, a total of 5 genes rs395561 (EFNA5), rs2690262 (UNCX), rs9720889 (SCXB), rs10992979 (BARX1), rs7127817 (NCAM1), with significant association to functional connectivity. Identification of patients that will not respond to standard therapy is important as these patients could be enrolled into biological therapy at early stages.

J15.08
Anti-TNF treatment response in Crohn's disease patients is associated with genetic polymorphisms and gene expression

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Anti-tumor necrosis factor (anti-TNF) agents are successful therapies in Crohn's disease (CD) however inadequate response occurs in up to 30% of patients treated. We have already identified strong association with anti-TNF response for ATG16L1 SNP rs10210302 and additional SNPs in 7 out of 31 tested CD associated genes. To improve predictive model of genetic markers for anti-TNF response we further investigate expression of 12 CD associated genes in well characterized cohort of 102 Slovenian CD patients treated with anti-TNF drug adalimumab (ADA) for which response was defined according to inflammatory bowel disease questionnaire (IBDQ) and C-reactive protein (CRP) 4, 12, 20 and 30 weeks after treatment, representing 570 RNA samples. In addition we tested if 22 SNPs previously associated with anti-TNF response in related autoimmune diseases (ankylosing spondylitis, rheumatoid arthritis, multiple sclerosis) could predict response in CD patients receiving ADA. We found strong association of SNP rs2275913

in IL17A gene with response to ADA in CD patients (p=0,006) where patients with GG genotype responded better compared to patients with AA genotype. Further we found strong associations between anti-TNF response and SNPs in genes MIF, TNFα, TNFRSF1A and CD14. We also found that expression of two selected CD associated genes, ATG16L1 and SLC22A5, correlates with response to ADA treatment, where expression of ATG16L1 and SLC22A5 genes was increased in responders compared to non-responders (p=0,014 and p=0,042, respectively). To predict response to anti-TNF therapy, SNPs as well as gene expression serve as promising markers for future personalized therapy.

J15.09

Janus Kinase 2 V617F mutation as genetic biomarker used for diagnostic criteria of myeloproliferative neoplasms: comparison of two methods proposed for clinical approaches

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Introduction: Janus kinase 2 (JAK2)V617F mutation is associated with myeloproliferative disorders. Its blood clonal level may be considered a diagnostic criteria of essential thrombocythemia, polycythemia vera, primary myelofibrosis and other disorders. The early detection of this mutation in suspected patients may provide important information regarding the prediction of the incidence of leukemic transformation and possible interventional schemes.
Materials and methods: We analyzed 30 DNA samples from patients with MPD diagnosis based on ARMS (amplification-refractory mutation system) -PCR method for Jak2V617F mutation detection. The i-densy (Japan Arkray 5600 system) based on automated digital HRM (High Resolution Melting Temperature) analysis using Q (quenching) Probes, that included direct blood DNA extraction, automated amplification and probe alignment, further specific amplicon analysis by melting temperature differentiation through rapid automated computerized data normalization and easily recording, as compared with ARMS-PCR approach involving time consuming DNA processing steps and finally an error prone electrophoretic estimation of mutant clone burden.
Results: HRM approach enabled a semiquantitative estimation of mutant clone burden which was not possible with ARMS method. Certain negative ARMS results turned in positive HRM results. Also ARMS method could not detect the benefic effect or inefficiency of treatment as HRM approach could do, by the variation of the mutant clone absorbance peak.
Conlcusions: HRM i-densy system proved a more sensible and semiquantitative measurement approach as compared with ARMS method based on easy sampling, automated DNA extraction, amplification and analysis steps and also on timing, repeatability and sensitivity in JAK2V617F mutation detection.

J15.10

Exploratory analysis of variants in HCN4 gene and in 3 SNPs of CYP3A4 gene for association with ivabradine reduction in heart rate.

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Objectives: Ivabradine, a selective bradycardic drug, inhibits the If. In patients with heart failure (HF), ivabradine reduce the risk of rehospitalization and mortality. The average heart rate (HR) reduction is 8-10 beats, but there is interindividual variability showed in clinical trials. The aim of the study is to identify variants associated with HR reduction produced by ivabradine in genes involved in the drug metabolism (CYP3A4) or related to the drug target (HCN4).
Methods: In an exploratory cohort (n=11), patients who started in ivabradine were genotyped and the HR reduction was studied.
Results: The mean HR reduction after the treatment was 18,10±12,26 bpm. The reduction of HR was ≥15bpm in 3 patients and >5 and <15bpm in 7 patients. Four synonymous variants, L12L, L520L, P852P, and P1200P, were detected in HCN4 gene (Frequency= 0.045, 0.045, and 0.681, respectively). Moreover, the CYP3A4*1F and CYP3A4*1B were found in one patient each and the CYP3A4*1G was presented in 3 patients.
Conclusions: This is the first study using an exploratory pharmacogenetic approach that try to explain the interindividual variability in ivabradine HR reduction. However, more research must be done to determine the role of variants in HCN4 and CYP3A4 genes in the response to ivabradine.
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J15.11

Association of obesity susceptibly gene variants with obesity and related traits in Tatars women

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Genome-wide association studies have revealed gene variants associated with obesity and related traits (anthropometric, biochemical parameters); but have no investigations among Tatars, which differ in the distribution of alleles several genes from both the populations of Asia and Europe. We examined the associations of metabolic traits and obesity related locis with risk of obesity. 10 SNPs (SEC16 rs10913469, FTO rs9939609, rs7202116, rs9930506, MCR4 rs12970134, rs17782313, TMEM18 rs2860323, rs6548238, CRP rs1130864, LIPC rs1800588) were genotyped by TaqMan and by PCR and RFLP (restriction-fragment-length polymorphism). Genotyping was performed in 460 Tatars women aged 48.17±10.52 years.
The FTO rs9939609 variant was associated with obesity (OR 1.50, CI95% 1.06-2.12, P=0.021). Allele C TMEM18 rs2860323 was related to obesity (OR 2.22, CI95% 1.35-3.55, P=0.0011) and allele C TMEM18 rs6548238 was associated with obesity (OR 1.64, CI95% 1.12-2.40, P=0.013). SEC16B rs10913469 and MCR4 rs12970134 were associated with obesity (OR 3.23, CI95% 1.83-5.71, P<0.0001 and OR 3.81, CI95% 1.16-12.50, P=0.019, respectively). The CRP rs1130864 variant was associated with obesity (OR 1.54, CI95% 1.10-2.16, p=0.012).
The fasting serum insulin, OGTT, hs-CRP, serum triglyceride levels, HDL and LDL cholesterol assessed by general linear mode. Mean levels of fasting serum insulin and C-peptide were higher in TT genotypes versus CC/CT CRP rs1130864 (P=0.009 and P=0.006). Mean levels of glucose at 2 h and HOMA-IR were higher in TT versus CC/CT LIPC rs1800588 (P=0.006 and P=0.03). The rest clinical and biochemical parameters of metabolic disorders did not differ depending on the polymorphic loci genes.
The work was done under supporting Russian Humanitarian Scientific Fund №13-06-00101.

J15.12

Genetic analysis of the rs2298881 polymorphism in the ERCC1 gene in Slovenia

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Background: The excision repair cross complementation group 1 (ERCC1) protein could be a risk factor for different cancer types and could influence patients' response to platinum-based cancer chemotherapy. The aim of the present study was to genotype the rs2298881 polymorphism in the ERCC1 gene in the general Slovenian population.
Patients and methods: In total, 88 healthy individuals were included in the study. Genomic DNA was extracted from whole-blood samples using QIAamp® DNA Blood Midi kit. Genotyping of the rs2298881 ERCC1 polymorphism was done using TaqMan® SNP Genotyping Assay C_16191190_10 and qPCR LightCycler 480 System II.
Results: The rs2298881 ERCC1 genotype frequencies in the general Slovenian population were 14.7% (13/88) and 85.3% (75/88) for AC and AA genotypes, respectively. No CC genotype was identified in our study sample.
Conclusions: In order to establish more effective strategies for prevention and treatment of cancer, the rs2298881 ERCC1 genotype distribution should be further investigated in cancer patients, especially those receiving platinum-based chemotherapy. According to the present as well as to some previous studies, the CC genotype might be characterized as the "risky genotype".

J15.13

The contribution of the immune response genes polymorphisms to chronic obstructive pulmonary disease in Russia

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Chronic obstructive pulmonary disease (COPD) is a complex chronic inflammatory disease of the respiratory system affecting primarily distal respiratory pathways and lung parenchyma. This work was designed as a association study aimed at investigating the association of COPD with JAK1, JAK3, STAT1, STAT3, NFkB, TNFA, LTA, IL6, IL17A, ADIPOQ, ADIPOR1 in a Tatar population from Russia.
Methods: 15 SNPs (rs310216, rs3212780, rs12693591, rs2293152, rs28362491, rs1800629, rs909253, rs1800795, rs4711998, rs1974226, rs3748067, rs1501299, rs266729, rs12733285, rs7539542 were genotyped by TaqMan assays in a case-control study (511 COPD patients and 508 control). Logistic regression was used to detect the association of SNPs. Linear regression were performed to estimate the relationship between SNPs and lung function parameters and pack-years.
Results: The rs1974226 IL17A (P=0.005 OR=2.22), rs1800795 IL6 (P=0.0018 OR=0.66), rs310216 JAK1 (P=0.0002 OR =1.70), rs3212780 JAK3 (P=0.0019, OR=1.39), rs2293152 STAT3 (P=0.036 OR=1.71), rs28362491 NFkB (P=0.015 OR=0.69), rs1800629 TNFA (P=0.0007 OR= 1.78) were significantly associated with COPD in additive model. The relationship between the rs3212780 JAK3 (P=0.0002), rs310216 JAK1 (P=0.0003), rs1501299 ADIPOQ (P=0.036) and emphysema risk was found. Chronic bronchitis phenotype was significantly associated wish rs1800629 TNFA (P=0.0005), rs1974226 IL17A (P=0.0074), rs2293152 STAT3 (P=0.0044), rs266729 ADIPOQ (P=0.019). The rs310216 (JAK1) and rs12693591 (STAT1) were associated with decrease of FEV1% predicted. A gene by environment interaction was observed for rs12733285 ADIPOR1 and rs266729 ADIPOQ and the smoking status (Pinteract = 0.003 and Pinteract =0.03). The rs1974226 IL17A, rs310216 (JAK1), rs3212780 (JAK3), rs2293152 (STAT3), rs28362491 (NFkB) loci were significantly associated with pack-years in smokers.
Our study confirmed the key role of systemic inflammation in development of COPD.

J15.14

Association of tuberculosis forms with polymorphisms of VDR gene among new cases

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Introduction. Environmental and lifestyle risk factors, genetically controlled host and bacterial factors contribute to tuberculosis (TB) development. Various publications show possible association of genetic polymorphisms of VDR gene with development of multidrug-resistant (MDR) TB of Beijing genotype.
Aim: To evaluate host-pathogen interactions in TB by comparing clinical-genetic features of TB patients with M. tuberculosis infection type (sensitive TB, mono-resistant TB, poly-resistant TB, MDR-TB)
Materials and methods. 80 patients with new pulmonary TB cases from Almaty, Kyzylorda and Kostanay regions were included in this study. Sample genotyping was done using Applied Biosystems 7900HT with TaqMan probes - rs2228570, rs7975232, rs731236 and rs1544410 for SNP markers FokI, ApaI, TaqI and BsmI of VDR gene, respectively. Drug resistance of M. tuberculosis to rifampicin, isoniazid, ethambutol and streptomycin was detected by absolute concentration method. Obtained results were confirmed by DNA sequencing of genes responsible for resistance to isoniazid - katG, ahpC-oxyR and fabG-inhA; rifampicin - rpoB; ethambutol - embB; and streptomycin - rpsL.
Results. Association of GG genotype of FokI with development of mono-resistant TB - 55,6 %; AC, AA and CC genotypes of ApaI (57,1%), TaqI (71,4%) and BsmI (71,4%) with development of poly-resistant form of TB was found among 4 groups. However, the results were not statistically reliable showing p-value 0,45; 0,84; 0,55 and 0,44 respectively.
Conclusion. No association between polymorphisms of VDR gene and infection with different forms of TB was found in our study. In order to confirm obtained results sample size should be increased and additional methods (MIRU-VNTR, spoligotyping) should be carried out.

J15.15

Association of CTLA4 polymorphisms as genetic risk factor in kidney transplant rejection

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Background: The most effective and common treatment for end-stage renal disease is kidney transplantation. CTLA4 may be a suitable candidate gene for studying allograft rejection.The aim of our study was to understand whether we can consider these two SNPs of CTLA4 gene as a risk factor of transplant rejection in Iranian population.
Materials and Methods: 169 kidney transplant recipients who underwent transplantation before 2010 were included in this study. The patients were classified into two groups according to Acute Rejection (AR) episodes. The -318C/T and +49A/G polymorphisms in CTLA4 gene were determined by RFLP.
Results: In +49 A/G polymorphism frequency of AG genotype was the same in both patients with and without history of rejection. None of those groups had homozygote genotype. Therefore, this polymorphism had no association with allograft rejection.In -318C/T both CT and TT frequencies among patients whose transplantations had been rejected
were lower than patients with normal outcome. Although that was not statistically significant. Compared to CC genotype as reference, TT genotype, caused 29% increase in the odds of rejection. However, this association was not significant (OR=1.29, p=1). **Conclusion:** The results of our study suggest that rs231775 (+49A/G) and rs5742909 (-318C/T) CTLA4 gene polymorphisms are not linked to acute rejection in the Iranian population who underwent kidney transplantation. So these polymorphisms cannot be considered as risk factors of acute allograft rejection in Iranian renal transplantation recipients.

J15.16

Investigation of CYP2C19 polymorphisms which effect clopidogrel resistance and development of stent thrombosis in stent implanted CAD patients

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Introduction: Stent implantation (ST) is one of the major treatment for individuals who have coronary artery disease (CAD. It is a devastating complication of coronary stent implantation. Clopidogrel is an antiplatelet prodrug used to treat or prevent atherothrombotic events after percutaneous coronary intervention (PCI). It decreases the incidence of coronary artery ST. Clopidogrel resistance can be due to a genetic variation in one of the cytochrome P450 hepatic enzymes, particularly CYP2C19. Patients with polymorphisms in this gene, have more adverse clinical events following PCI. Indeed, this gene has been independently shown to be associated with early ST. Therefore the aim of this study is to investigate the effect of CYP2C19 gene mutations to clopidogrel resistance and stent thrombosis in stent implanted CAD patients.
Material-Methods: 80 CAD patients who have PCI and receiving 75 mg/day clopidogrel were recruited to the study. They were divided into two groups. 40 patients who have stent thrombosis were recruited to the first group, others were recruited to the second group. After DNA was isolated from peripherial blood, CYP2C19 mutations were investigated with RT PCR. Results were evaluated statistically.
Results: 8 heterozygous and 1 homozygous CYP2C19*2 mutations and 1 heterozygous CYP2C19*3 mutations were found in the first group. CYP2C19*2 mutations were found statistically significant in the first group when they were compared with second group.
Conclusion: It is possible to recommend CAD patients for increasing the dosage of clopidogrel or using other efficient drugs for preventing stent thrombosis. Therefore genetic screening of CAD patients for CYP2C19 gene mutations will help the improvement of personilized therapy strategies.

J15.17

Cytokines genes in susceptibility to JIA in patients from Russia.

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Background: Some single nucleotide polymorphisms (SNPs) within cytokines and their receptors genes have been reported to be associated with juvenile idiopathic arthritis (JIA) and other autoimmune diseases in different populations.
Objectives: The aim of the study was to test the hypothesis that the TNFA, LTA, IL1B, IL2-21, IL2RA, IL6, IL10, MIF SNPs could underlie susceptibility to JIA or its subtypes in patients from Russia.
Methods: The TNFA (rs1800629), LTA (rs909253), IL1B (rs16944), IL2-21 (rs6822844), IL2RA (rs2104286), IL6 (rs1800795), IL10 (rs1800872), MIF (rs755622) SNPs was studied in 254 children with JIA and 204 healthy individuals from Bashkortostan, Russia using PCR-RFLP method and real-time PCR.
Results: LTA allele G (p=0,024), TNFA-LTA haplotype G-G (p=0,0009), IL6 genotype CC and allele C (p=0,026, p=0,021, correspondingly), MIF genotype GG and allele G (p=0,026, p=0,033, correspondingly) were significantly higher in JIA patients than in controls. Analysis stratifying by ILAR subtype showed that MIF genotype GG and allele G (p=0,005, p=0,013, correspondingly) were significantly higher than in controls in patients with RF-negative polyarthritis; LTA allele G (p=0,019), TNFA-LTA haplotype G-G (p=0,0016), IL6 genotype CC and allele C (p=0,005, p=0,012, correspondingly), IL10 genotype CC and allele C (p=0,019, p=0,022, correspondingly) - in patients with persistent oligoarthritis; LTA genotype AG (p=0,018), TNFA-LTA haplotype G-G (p=0,018), IL1B genotype TT (p=0,038), MIF genotype GG and allele G (p=0,038, p=0,050, correspondingly) - in patients with enthesitis-related arthritis.
Conclusions: Our data demonstrate the association of the TNFA (rs1800629), LTA (rs909253), IL1B (rs16944), IL6 (rs1800795), IL10 (rs1800872), MIF (rs755622) polymorphic variants with increased risk of JIA and/or its subtypes in patients from Russia.

J15.18

VDR gene polymorphisms impact on anemia at 2 week of anti-HCV therapy: a possible mechanism for early RBV-induced anemia.

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Vitamin D receptors (VDR) bind calcitriol and modulate several physiological systems, through genomic and non genomic pathways. Calcitriol stimulates store-operated channels Ca2+ influx by translocation of the caveolar VDR to the plasma membrane. Intracellular Ca2+ levels in erythrocytes control biophysical properties and an increase in its concentration can deregulate membrane composition, cell volume, glycolytic enzymes regulation, redox state and cell clearance.
We evaluated the role of single nucleotide polymorphisms in ITPA, CYP27B1, CYP24A1 and VDR genes in the prediction of ribavirin-induced anemia, in HCV-1/2/3/4 patients at 2 and 4 weeks of treatment.
Two hundred and twenty five patients treated with ribavirin and pegylated-interferon-α were genotyped by real-time PCR.
BMI at baseline>30 Kg/m2 (p=0.013, OR:10.95, IC95%:1.66-74.21), ALT at baseline>37 IU/L (p=0.020, OR:0.26, IC95%:0.09-0.81) and VDR BsmI AA profile (p=0.003, OR:5.09, IC95%:1.72-15.05) were anemia predictive factors at 2 weeks of therapy. At week 4, ITPA rs6051702 AC/CC profile (p=0.001, OR:0.19, IC95%:0.07-0.51) was the only factor able to predict this side effect.
BsmI AA genotype is a predictive factor of 2 weeks anemia and it could be related to a VDR enhanced activity, thus an increased calcium influx, resulting in the deregulation of the Ca2+-dependent signaling, which can lead to erythrocytes hemolysis. This rapid mechanism could be responsible of the early anemia development.
These results indicate for the first time the strong, significant and independent role of VDR in the early development of ribavirin-induced anemia and confirm the ITPA function in the prediction of anemia at week 4.

J15.19

microRNA profiling recognizing CML phases` pathophysiology

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Chronic myeloid leukemia (CML) is a myeloproliferative disorder of hematopoietic stem cells carrying the Philadelphia (Ph) chromosome and an oncogenic BCR-ABL1 fusion gene. Oncogene and tumor suppressor microRNAs (miRNAs) are important regulators of transcription in hematopoiesis. Their expression deregulations were described in association with pathogenesis of chronic myeloid leukemia (CML), although they are still not fully understood. Our study was performed to assess and validate selected group of miRNAs in peripheral blood of patients for early detection and confirmation of found differences between patients with clinically defined CML phases and patients with uncorfirmed diagnosis. On mononuclear cells isolated from peripheral blood of CML positive versus CML negative patients, miRNA microarray analysis was performed. Selected miRNAs were validated by qRT-PCR. Using microarrays we identified differential expression profiles of 46 upregulated and 24 downregulated miRNAs in suspect CML patients at diagnosis distinguishing CML positive patients and patients with uncorfirmed diagnosis. Significantly increased expression of miR-17, miR-18a, miR-19a, miR-20a, miR-21, miR-27a and miR-155 in CML positive compared to CML negative patients and healthy individuals was confirmed by real-time quantitative PCR.
This study confirms the involvement of miR-17, miR-18a, miR-19a, miR-20a, miR-21, miR-27a and miR-155 in CML pathogenesis and suggests their possible clinical utilization as biomarkers for early recognition and confirmation of CML diagnosis.
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J15.20

TNF-α GENE POLYMORPHISMS AND JUVENILE IDIOPATHIC ARTHRITIS: INFLUENCE ON DISEASE OUTCOME AND THERAPEUTIC RESPONSE

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Objective: To investigate the genetic contribution of TNF-α gene polymorphisms on the disease course and therapeutic response in patients with juvenile idiopathic arthritis(JIA).
Methods: 74 Caucasian patients with JIA were recruited with a control group of 77 healthy children. DNA was extracted for analysis of TNF-α gene promoter polymorphisms at position -163, -244, -238, -376 and -308.
Results: No SNPs at position -163 we observed while were observed only SNPs at position -244 and -376 in the controls. No differences were observed in the prevalence of SNPs at -238 and -308 between JIA and controls. In JIA patients no significant differences were observed between the -238 and -308 G/A genotypes and different disease phenotypes. We observed a significant lower disease activity expressed in the carriers of -308 GG genotype with respect to GA and AAgenotypes after 6 (p=0.008 p=0.013 respectively) and 12 months of disease (p=0.02 p=0.08 respectively). Also the -238 GG genotypes showed a better disease course after12 months of disease. Moreover the -238/-308 GG genotypes presented the higher reduction of disease activity both after 6 (p<0.01vs GA and p<0.01 vs AA) and 12months from baseline (p<0.01vs GA and p<0.01 vs AA). After 12 months of biologic therapy, a significant higher disease activity was observed in patients with genotype -308AA respect to both GA (p=0.012) and GG (p=0.016).
Conclusions: JIA patients carrying the TNF-α -308 GA/AA and -238 GA genotypes are associated with a worse prognosis and with a lower response to anti-TNF-α drugs.

J15.21

The effect of polymorphisms in DNA repair genes and carcinogen metabolizers on leukocyte telomere length

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Introduction: Smoking implies exposure to carcinogenic agents that causes DNA damage, which could be suspected to enhance telomere attrition. To protect and deal with DNA damage cells possess mechanisms that repair and neutralize harmful substances. In this study we evaluated the effect of genetic polymorphisms in DNA repair genes and carcinogen metabolizers on leukocyte telomere length (LTL) in a cohort of healthy smokers.
Material and Methods: LTL and six genetic polymorphisms in CYP1A1 (Ile462Val), XRCC1 (Arg399Gln), APEX1 (Asp148Glu), XRCC3 (Thr241Met)

and XPD (Asp312Asn; Lys751Gln) were analyzed in 145 healthy smokers in addition to smoking habits. Genetic markers were determined by RT-PCR in blood samples. A logistic regression analysis adjusted for age, smoking history and gender was performed.
Results: Analysis showed an association between XRCC1 399Gln allele and shorter telomere length (OR=5.03, 95% CI=1.08-23.36). There were not association between the rest of polymorphisms analyzed and shorter telomere length, APEX1 (Asp148Glu) (OR=7.39, 95% CI=0.92-59.43), XRCC3 (Thr241Met) (OR=1.93, 95% CI=0.38-9.69), XPD (Asp312Asn) (OR=1.53, 95% CI=0.50-4.69), XPD (Lys751Gln) (OR=1.2, 95% CI=0.38-3.77) and CYP1A1 (Ile462Val) (OR=1.96, 95% CI=0.77-4.91).
Conclusions: Our data provide evidence that XRCC1 399Gln allele appears to confer sensitivity to the effects of tobacco compounds on LTL. Continuous exposure to tobacco could overwhelm the DNA repair machinery, making the effect of the polymorphisms that reduce repair capacity more pronounced. Analyzing the function of smoking-induced DNA-repair genes and LTL is an important goal in order to identify therapeutic targets to treat smoking-induced diseases.
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J15.22
Design and implementation of novel multiplex PCR assay to genotype EGFR mutations in patients with non-small cell lung carcinoma.

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Introduction: Lung cancer is the leading cause of cancer death worldwide. Approximately 80% of lung cancers are grouped as Non Small Cell Lung carcinoma (NSCLC). Mutations in Epidermal Growth Factor Receptor gene (EGFR) have been associated with improved response to tyrosine kinase inhibitors in patients with NSCLC. Two most common mutations, exon 19 deletion (E746-A750del) and exon 21 L858R Single Nucleotide Polymorphism (SNP) account for almost 90% of all EGFR mutations.
Objective: The aim of this study is to design and implement a new PCR assay for the screening of the two most common therapy-related EGFR mutations.
Methods: A Multiplex allele specific PCR assay was designed to detect the most common exon 19 deletion (E746-A750del) and exon 21 L858R SNP. Allele specific tetra primes were designed. To validate the assay for clinical diagnostics DNA was extracted from Formalin Fixed Paraffin Embedded (FFPE) tumor sample using QIAamp DNA FFPE Tissue Kit,
Results: PCR reactions were carried out in a single tube after optimization of the PCR conditions such as primer concentration, annealing temperature, and MgCl2 concentration. The designed assay provided the predicted amplification pattern for both mutant and normal genotypes
Discussion: The allele specific multiplex PCR assay we designed is fast and easy to perform in routine diagnostic laboratory compared with other proposed method for detection of EGFR mutations in NSCLC. PCR reaction could be carried out in a single tube with inexpensive reagents using standard PCR techniques which can be easily implemented at any clinical laboratory. This assay is sensitive and cost effective for initial EGFR testing.

J15.23
HLA-B*57:01: the Direct-PCR as fast and low-cost technology for pharmacogenetic screening

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The extensive knowledge of the interindividual variability of the human genome paved the path for the development of the personalized medicine, based on the employment of personal genomic signatures as predictive, diagnostic and pharmacogenetic biomarkers (PGBMs). One of the most successful applications of PGBMs into the clinical practice is the genetic screening for the HLA-B*57:01 allele, which is strongly associated with a high risk of Hypersensitivity Reaction (HSR) to Abacavir (an antiretroviral drug for HIV treatment). Given the clinical utility of HLA-B*57:01 screening in decreasing the incidence of drug HSR, the test became mandatory prior Abacavir administration. Taking into account the limits of the current typing methodologies, the aim of our work was the development and validation of a fast, easy to use and inexpensive molecular approach for the HLA-B*57:01 allele detection. In our assay the DNA extraction and amplification are combined in one single step and performed directly on the biological sample (Direct-PCR), without needing any extraction/purification and sequencing procedures. The Direct-PCR phase consists of the amplification of HLA-B*57 region and allows to discriminate HLA-B*57-negative patients (~90%), who are eligible for Abacavir therapy. The remaining HLA-B*57-positive individuals (~10%) are further typed by Nested PCR, in order to recognize HLA-B*57:01-positive patients (7%) and prevent the dangerous administration of Abacavir. Among the different advantages, the low-cost technology required for the Direct/Nested-PCR assay promotes the exportation of the HLA-B*57:01 pharmacogenetic test in Low and Middle Income Countries, where current genotyping methodologies are too expensive or not available.

J15.24
Bytes, human genotypes, and clinical phenotypes: case examples highlighting tools, methodologies, challenges, and successes from our adult and paediatric genomic medicine clinics.

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Introduction: Despite significant advances over the last decade in our understanding of the basis of disease, the cause underpinning the majority of human disorders remains fully or partially unknown. Identification of molecular changes provides a unique opportunity to understand their role in health and disease, and in a clinical setting to apply that understanding to prevention, diagnosis, and treatment.
Materials and Methods: The advent of high-throughput genome wide sequencing has significantly altered how genetics is practised in the clinic. We have been applying clinical genome wide sequencing (GWS; encompassing both whole genome and whole exome) within our genomic medicine clinics at MCW and CHW for more than 4 years.
Results: With application of both whole genome and whole exome sequencing technology and development of advanced informatics solutions we have been able to successfully diagnose more than a third of our patients. This includes many who would not have received a diagnosis through the previous standard of care testing. The diagnostic success rate is significantly higher with whole genome sequencing compared to whole exome.
Conclusions: This talk will provide a discussion of issues relevant to clinical application of genomics. Topics will include the development of informatics tools, methodologies, and decision making processes. Vignettes based on findings from particular cases will be presented to demonstrate what we have learnt over this time as well as to highlight ongoing challenges and future tasks.

J16.01
Molecular diagnosis of Eye disorders: Pitfall and benefits of Next Generation Sequencing

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Human genome project opened a door to fast and accurate diagnosis of disorders. But sometimes molecular detection of diseases such as eye disorders is very challenging, because of involving numerous genes. For instance, there are more than 200 known genes causing retinopathy. Hunting the right gene in these complex disorders is like finding the needle in the haystack. Nevertheless, modern sequencing platforms known under collective name Next generation sequencing or NGS is a huge help to solve this problem. One of great benefits of NGS is to see a list all candidate genes including disease causing gene mutations. On the other hand, accuracy of NGS is extremely depending on frequency and length of reads. So, NGS platforms that produce and sequence longer length show fewer errors. However, sequencing of terminals is generally a weak point in all NGS platforms. We experienced due analysis numerous NGS data that results are sometimes not valid (at least in most cases) within repeat sequences or stretches of single bases. For instance, in a Retinitis Pigmentosa (RP) case we found a homozygous insertion of single C within repeated sequence CCCCCCC in the NR2E3 gene that

is known being responsible for Retinitis Pigmentosa type 37. But, Sanger sequencing was negative for reported insertion. However, to avoid similar pitfalls we suggest using two different NGS method such as Ion proton and High seq, which is in large scale samples very expensive.

J16.02

Proteomic output of a whole chromosome aneuploidy: a systems biology study using fibroblasts from trisomy 21 discordant monozygotic twins

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Introduction: Down's syndrome commonly results from total trisomy of chromosome 21 (T21). To study the dysregulated gene expression without the noise of genomic variability, we previously used fibroblasts from a pair of monozygotic twins discordant for T21 and revealed the transcriptomic dysregulation between twins is organized in chromosomal domains[1]. These findings lead us to further investigate the translational profiles due to the supernumerary T21 in fibroblasts.
Materials and Methods: We used SWATH-MS[2], a novel mass spectrometry technique providing unprecedented reproducibility and quantitative accuracy for proteomic analysis. The primary fetal skin fibroblasts collected from the T21 discordant twins and fibroblasts from 11 unrelated T21 and 11 control individuals were analyzed.
Results: We reproducibly quantified 3,548 unique proteins assigned by 32,753 peptides between the T21 and Normal twin (R=0.979 between bio-replicates). The protein-transcript quantitative correlation was ~0.40. The detectable 32 proteins encoded in chromosome 21 on average harbored a higher expression in T21, whereas globally only ~40% of the proteome showed higher expression in T21. Interestingly, 291 proteins (most are non-chr21 encoded) were significantly up-regulated in T21, while only 63 proteins were significantly down-regulated. Moreover, GO processes such as translational initiation and protein transport were significantly enhanced in T21 proteome (P<0.0001), indicating the translational regulation resulting from the extra chromosome is remarkably extensive.
Conclusions: We discovered prevalent proteomic consequences of T21 by SWATH-MS. Further investigations are ongoing to increase the proteome coverage and to understand the biological correlations between the transcriptome and proteome.
[1]Letourneau, A., et al. Nature (2014).
[2]Gillet, L., et al. Molecular & cellular proteomics (2012).

J16.03

Unique identifiers downstream from the TATA Box in human genes and human DNA embedded viral genomes

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The human genome consists of approximately 21,000 protein coding genes. We hypothesized there exists an organized means for transcription machinery to locate a specific gene. Transcription Binding Protein (TBP) is an initial transcription factor that binds to DNA to commence assembly of the transcription complex. TBP attaches to the DNA in the vicinity of the TATA box upstream from the transcription start site (TSS). Frequently there exists 25 nucleotides between the TATA box and the TSS. 25 nucleotides would be sufficient to uniquely identify 200,000 differing genes for 5 billion species. It is estimated 25% of human genes have a TATA box upstream from the TSS. We hypothesized there may be a unique numbering system associated with a subset of genes, and DNA may be divided into executable genes (locatable gene associated with a unique identifier) and follower genes (transcribed automatically following transcription of an executable gene). Eight human genes with a TATA box and four viral genomes which embed in human DNA are reported. The 25-nucleotide sequence downstream from the TATA box was converted to the numbering system a=0, g=1, c=2, t=3 as published in ESHG 2014 Abstract J16.03. NCBI BLAST analysis determined the complete TATA+25-nucleotide unique identifiers associated with the reported genes were not otherwise found intact in the human genome. More extensive study is forthcoming. The unique identifiers associated with embedded viral genomes may be utilized as inimitable targets for future anti-viral therapies.

Gene Unique Identifiers

Gene	25 Nucleotide Sequence Position	TATA-25 Unique Identifier	BLAST Closest Match 5'-3'	Numerical Conversion of Unique Identifier Using a=0, g=1, c=2, t=3
INS	2160-2184	TATA aagccagcgggggcccagcagccct	18/29	00122012111112220120122 23
LEP	4975-4999	TATA agaggggcgggcaggcatggagccc	19/29	01011112111201120311012 22
RMRP	134504-134528	TATA aaatactactctgtgaagctgagga	16/29	00030230232313100123101 10
TPI1	573-597	TATA taagtgggcagtggccgcgactgcg	15/29	30013111201311221210231 21
AGT	441-465	TATA aataggggcatcgtgacccggccgggg	15/29	00301112032131022211221 11
hGH	126-150	TATA aaaagggcccacaagagaccagctc	15/29	00001112220200101022012 32
NOS2A	8271-8295	TATA aatacttcttggctgccagtgtgtt	15/29	00302332331123122013131 33
TNF	1717-1741	TATA aaggcagttgttggcacacccagcc	16/29	00112013313311202022201 22
HIV HXB2	431-455	TATA agcagctgctttttgcctgtactgg	20/29	01201231233333122313023 11
HSV-1 gC	96,145-96,169	TATA aattccggaaggggacacgggctac	18/29	00332211001111020211123 02
VZV ORF21	30,734-30,758	TATA aagttaagtcagcgtagaatatacc	16/29	00133001320121301003030 22
Smallpox	247-275	TATA cttttaattgaacaaaagagttaag	20/29	23333003311002000010133001

J16.04

mtMart - a new database for effective human mitogenomic data analysis

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Human mitogenomic data is continuously accumulating while effective methods of massive information storage and management lag behind. Publicly available databases of human mtDNA are either not regularly updated or lack functional tools for appropriate data parsing. For instance, HvrBase++ and mtDB databases, launched in 2006 have not been updated since 2007. Conversely, the Phylotree and Mitomap databases though updated regularly, lack functional tools for data handling, reducing its efficacy.
Here, we introduce the mtMart - a novel manually curated database for complete human mitochondrial genomes that is intended to significantly facilitate effective management of large-scale mitogenomic data.
The database is designed applying the MySQL open-source relational database management system. Its functional features are implemented in PHP and JavaScript (jQuery) programming languages. Using NCBI API, the database retrieves the information on mitogenomes accession number, complete mtDNA sequence, and the publication where the molecule was first described, subsequently storing the data in internal memory. Afterwards, mtMart semi-automatically sends the sequence information to the Haplofind on-line application to assign haplogroups according to Phylotree 16, using the RSRS reference sequence. The data on population and geographic region is manually appended to the database, thus ensuring high quality of stored information. mtMart provides the users with customizable sorting, searching and downloading functions. Moreover, for population/geographic region and haplogroup data, mtMart automatically generates an Arlequin .arp input file with haplogroup frequency data.
Though the mtMart database is available through http://genebank.hol.es/genebank/, its development is still in progress.

J16.05

Identification of a new IGL gene fragment in human RFPL2 gene

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There are a lot of orphons (duplications of Ig or TCR gene fragments containing V-, D- or C-segments) in human genome outside Ig and TCR loci. Some of them can be find in protein-coding genes. For example, IGLCOR22-1 orphon (length is 316 bp) is located in RFPL2 gene (in the 4th intron) and has 83 % identity with immunoglobulin lambda light chain C region (IGLC2) gene.
Here we report that 1947 bp RFPL2 gene fragment with IGLCOR22-1 orphon has 81 % (1585/1947) identity with immunoglobulin lambda light chain region containing IGLJ5 and IGLC5 genes. These data demonstrate: a) IGL gene fragment in RFPL2 gene is 6 times bigger than reported earlier and contain homologues of IGLJ5 and IGLC5 (not IGLC2); b) in the human genome there are duplications of J-segments of IGL (not only V- and C-segments); c) appearance of close structural analogs of recombination signal sequences (cRSS) in protein-coding genes can be explained not only random nucleotide combinations and repeats but also by duplications of Ig, TCR gene fragments.

J16.06

Bioinformatics analysis of mi-RNA motifs distribution in cytokine genes and their surroundings

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Cytokine production is the one of the basic processes in immune response, inflammation and hemopoiesis. Understanding of cytokine genes regulation and in particular mi-RNA regulation could lead to effective and targeted therapies of many diseases.
Based on the findings that genome functioning is tightly connected with its structure, we have conducted the bioinformatics analysis of mature mi-RNA and pre-mi-RNA motifs distribution in 16 cytokine genes and their surroundings to discover common and specific patterns of mi-RNA localization.

Full sequences were obtained from NCBI data base using E-utilities API. Mi-RNA sequences were taken from the miRBase release 21. Motif search was carried out with MEME Suite program package. The results were filtered to yield only those matches with 85% identical nucleotides.
The entire set of DNA sequences 16 cytokine genes and their surroundings contained 6007 motifs of 19-23 nucleotides homologous to mature mi-RNA sequences and 873 motifs of 68-72 nucleotides homologous to pre-mi-RNA sequences. MiR-466, miR-5096, miR-619 and miR-1273 were prevalent motifs in studied DNA sequences. Prevalent motif type and the density of motifs distribution varied from gene to gene. These results can be discussed as a background to the search of new targets for diagnostics and therapy.
This study was supported by the federal assignment № 6.98.2014/ from Russian Ministry of Science and Education. Analytical work was carried out on the equipment a Center for collective use of Southern federal university „High Technology", grant #RFMEFI59414X0002.

J17.01

The role of DNMTs genes expression in pomalidomide-based epigenetic effect on U266 Myeloma cell line in invitro

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Multiple myeloma (MM) is clonal B-cell malignancy characterized with progressive proliferation of malignant plasma cells and accumulation of monoclonal immunoglobulin (M-spike) in blood and urine. Pomalidomade is immunomodulatory agent which have potentially suppress myeloma cell progression, especially in drug resistant cases. As epigenetic modifications have important role in gene regulationand because of revealing role of DNMT1 overexpression in myeloma pathogenesis, in this study DNMT1, 3a and 3b genes expression of U266 myeloma cell line treated with pomalidomide have been evaluated. In this study after treatment of U266 cells with 1 µM pomalidomide for 16 and 48 hours, total RNA extraction and cDNA synthesis was performed. Gene expression of DNMT1, 3a and 3b has been evaluated using real time PCR technique. The result of this study show that pomalidomide can downregulates the expression of DNMT1, 3a and 3b in 48 hours of treatment as 0.49, 0.58 and 0.55, respectively as comparing with untreated control ($p<0.05$). Based on these results we conclude that pomalidomide has derired effect on epigenetic modification by downregulation of DNMTs genes expression and has been considered as an effective drug for inhibition of myeloma proliferation.

J17.02

Detection of SHOX2 gene DNA methylation in bronchial lavage and blood plasma in the lung cancer patients

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Methylation of the cytosine residues within the CpG dinucleotides plays an important role in the fundamental cellular processes, human diseases and even cancer. The DNA methylation represents a very stable sign and therefore may be used as a valuable marker for cancer screening. Epigenetic cancer biomarkers are independent of classical morphology and thus show extensive potential to overcome the limitations of cytology. Several epigenetic cancer markers have been reported to be detectable in body fluids such as bronchial aspirate, sputum, plasma and serum.
Short stature homeobox gene 2 (SHOX2) encodes a homeo-domain transcription factor, which has been identified as a close homologue of the SHOX gene and both genes are involved in skeletogenesis and heart development. Methylation of SHOX2 gene has been shown to be present at high prevalence in carcinomas of lung, however may also be used to identify other tumour entities.
In the presented study, we have compared suitability of two types of material associated with lung cancer for the detection of SHOX2 methylation. Using the appropriate DNA purification and quantitative real-time PCR kits (Epigenomics), we have tested SHOX2 methylation in samples of bronchial lavage and blood plasma from the same patients. Mutual correlation of these results as well as correlation with the clinical outcome were assessed and are reported.

J17.03

CpG island methylation status of estrogen receptor alpha in a mexican breast cancer group women

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Introduction: Breast cancer (BC) involves hormonal and epigenetic risk factors. Hormone resistance, due to hyper-methylation of the ESR1 (ERa gene) promoter, may occurs. This epigenetic alteration, is known to be one of the mechanisms by which the expression of ERa is suppressed. The goal of the present study was to determine the methylation status of the promoter A CpG island of the cancer-related gene ESR1 in a mexican woman group. Materials and methods: CpG island of the ESR1 promoter A, was detected by semi-quantitative PCR. A total of 51 patients were included: 17 controls (non breast cancer women), 17 cases ERa positive (<50%) and 17 cases ERa positive (>50%). Results: There was no difference in the prevalence of the DNA methylation of ESR1 gene between the three studied groups. Our population shown a parametric distribution. Conclusions: Our preliminary findings suggested that aberrant methylation of ESR1 occurs frequently in mexican women. On the other hand, although the methylation status didn´t show a statistical difference among case and control groups, future studies in the studied population must include an extensive clinical parameters and the examination of the methylation of more genes are needed.
Grant references:
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J17.04

A large-sized genomic deletion at the DYSF locus deregulates Retinoic Acid-dependent CYP26B1 expression and causes Antley Bixler Syndrome

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Antley Bixler Syndrome (ABS) constitutes a very rare developmental syndrome characterized by craniosynostosis and radiohumeral synostosis which is genetically determined by mutations in the cytochrome P450 oxidoreductase gene (POR), the Fibroblast Growth Factor Receptor 2 gene (FGFR2) or the cytochrome P450 gene CYP26B1. Interestingly, clinical manifestations together with the genes involved, pinpoint on the retinoic acid (RA) signalization pathway involvement. The CYP26B1gene, which maps on minus strand of chromosome 2, is located 444kb downstream of the DYSF gene. Syntheny and relative gene positions are highly conserved through evolution.
Based on the specific exploration of one patient presenting with combined clinical manifestations of ABS and Miyoshi myopathy (MM), we report that DYSF contains regulatory sequences controlling CYP26B1 expression that, when deleted, lead to ABS by a long distance position effect. Strikingly, activation of CYP26B1 expression by RA is greatly reduced in lymphoblasts from one patient carrying a large internal deletion at the DYSF locus as compared to controls. Thus, the genomic DYSF deletion in this case not only affects dysferlin protein function but also CYP26B1 gene expression explaining the clinical outcomes for this patient. Altogether, these data underline the importance of searching for DYSF locus microdeletions in ABS patients without mutations in classically linked genes. Even in high-throughput sequencing era, it is worth to look for rearrangements causing positional effects, in patients showing combined clinical signs.

J17.05

Histone demethylase KDM2B inhibited the chondrogenic differentiation potentials of stem cells from apical papilla

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Objective: Mesenchymal stem cells (MSCs) are a reliable resource for tissue regeneration, but the molecular mechanism underlying directed differentiation remains unclear; this has restricted potential MSC applications. Histone methylation, controlled by histone methyltransferases and demethylases, may play a key role in MSCs differentiation. Previous studies determined that KDM2B can regulate the cell proliferation and osteo/dentinogenic differentiation of MSCs. It is not known whether KDM2B is involved in the other cell lineages differentiation of MSCs. Here we used the stem cells from

apical papilla (SCAPs) to study the role of KDM2B on the chondrogenic differentiation potentials in MSCs.
Methods: Gain- and loss-of-function assays were applied to investigate the role of KDM2B on the chondrogenic differentiation. Alcian Blue Staining and Quantitative Analysis were used to investigate the synthesis of proteoglycans by chondrocytes. Real-time RT-PCR was used to detect the expressions of chondrogenesis related genes.
Results: The Alcian Blue staining and Quantitative Analysis results revealed that overexpression of KDM2B decreased the proteoglycans production, and real-time RT-PCR results showed that the expressions of the chondrogenic differentiation markers, COL1, COL2 and SOX9 were inhibited by overexpression of KDM2B in SCAPs. On the contrary, depletion of KDM2B increased the proteoglycans production, and inhibited the expressions of COL1, COL2 and SOX9.
Conclusions: These results indicated that KDM2B is a negative regulator of chondrogenic differentiation in SCAPs and suggest that inhibition of KDM2B might improve MSC mediated cartilage regeneration.

J17.06

Gene-gene interactions in smoking and non-smoking adolescents with asthma

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Asthma is a common condition with complex etiology. Susceptibility to asthma is due to interaction of genetic and environmental factors.The impact of smoking on asthma risk was analyzed in adolescents from Aguinskiy Buryat district of Zabaykalskiy region (Western Siberia, Russian Federation). An association study by genotyping for 11 polymorphisms of 9 genes in patients with asthma (49 smokers and 55 non-smokers) and 75 healthy controls (75 smokers and 75 non-smokers). The average age is 14,5±0,90y. The structure of asthma genetic predisposition differs between smoking and non-smoking adolescents. Genotype TT (rs7216389; GSDMB) is a factor of an increased asthma risk in the both smokers (OR=8.2, 95%CI[3.6-18.8]; p<0,0001) and non-smokers (OR=3,26; 95%CI [1,6-6,8]; p=0,001). Asthma is associated with NOS3 (VNTR; p=0,03) and ADRB2 (Gln27Glu; p=0,03) genes in smoking patients, and with CHRNA5 (rs16969968; p=0,001), ADRB2 (Gln27Glu; p=0,01) and TH0I (STR; p=0,01) genes - in non-smokers. Analysis by multifactor dimentionality reduction (MDR) allowed to detect the more significant models of gene-gene interactions: GSDMB (rs7216389) x ADRB (Gln27Glu) x NOS3 (VNTR) x TH0I (STR) - for smokers; GSDMB (rs2305480) x GSDMB (rs7216389) x ADRB (Arg16Gly) x ADRB (Gln27Glu) x CHRNA5 (rs16969968) - for non-smokers. The most additional genetic attributive risk was 12.3% for the 4-locus genotype, GSDMB (rs7216389)*TT - ADRB (Gln27Glu)*CG - NOS2 (CCTTT)n*LL - TH0I (STR)*SS, in smoking asthma patients. Increased asthma risk genotypes revealed in non-smokers group were GSDMB (rs7216389*TT) -- CHRNA5 (rs16969968)*GG (OR=3,52; 95%CI [2,0-6,2], p<0,0001); GSDMB (rs7216389)*TT - ADRB (Arg16Gly)*AG - ADRB (Gln27Glu)*CC (OR=17,73; 95%CI [2,4-131,5]; p=0,0002) .
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J17.07

Aberrant methylation of Rap1Gap gene promoter in thyroid cancers

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Introduction: Thyroid carcinoma is the most common endocrine malignancy in worldwide. Hypermethylation of suppressor genes considered as the hallmark of cancers [1]. Rap1Gap as a suppresser gene is implicated in the regulation of oncogenic pathways in thyroid carcinoma [2]. The objective of this study was to examine the DNA methylation pattern of Rap1Gap gene in thyroid carcinoma.
Methods: we analyzed 95 thyroid tumor samples including normal thyroid (28 cases), benign nodules (29 cases), papillary thyroid cancer (PTC) (29 cases), follicular thyroid cancer (FTC) (6 cases) and Anaplastic thyroid cancer (ATC) (3 cases) from Erfan grand hospital, Tehran, Iran. Rap1Gap gene expression was assessed using SYBR Green Real-Time PCR. CpG24 Island within Rap1Gap promoter region was selected; DNA methylation pattern was examined using methylation specific PCR (MSP).
Results: this study showed that Rap1Gap was frequently lost or downregulated in various types of tumors, particularly in the most invasive and aggressive forms of thyroid cancer. Downregulation could be related to promotor hypermethylation. DNA methylation was identified in 21% of normal tissues, 86% of benign nodules, 90% of differentiated tumors (PTC, FTC) and 100% of undifferentiated tumors (ATC).
Conclusion: the result of this study demonstrates that Rap1Gap is likely to serve as an important tumor suppresser gene in thyroid cells and its loss during aberrant methylation contributes to tumor progression and invasion.
References
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J17.08

Sequential changes in 5-hydroxymethylcytosine patterns during DNA methylation reprogramming in human zygotes and cleavage-stage embryos

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We have studied chromosome hydroxymethylation and methylation patterns in metaphase chromosomes from IVF-produced human triploid zygotes and cleavage-stage embryos. To obtain metaphase chromosomes, zygotes and embryos were treated with 0.1% colchicines, 0.9% sodium citrate and fixed with freshly prepared 3:1 methanol:acetic acid. Using indirect immunofluorescence, we have analyzed the localization of 5-hydroxymethylcytosine (5hmC) and its co-distribution with 5-methylcytosine (5mC) on the QFH-banded metaphase chromosomes.
In zygotes, 5hmC accumulates in both parental chromosome sets, but hydroxymethylation is more intensive in poorly methylated paternal set. In maternal set, chromosomes are highly methylated, but contain little 5hmC. Hydroxymethylation is highly region specific in both parental chromosome sets: hydroxymethylated loci correspond to R-bands, but not G-bands, and have well-defined borders, which coincide with the R/G-band boundaries. The centromeric regions and heterochromatin at 1q12, 9q12, 16q11.2 and Yq12 contain little 5mC and no 5hmC. We hypothesize that 5hmC may mark structural/functional genome 'units' corresponding to chromosome bands in the newly formed zygotic genome. Hydroxymethylation of R-bands in zygotes can be treated as a new characteristic distinguishing them from G-bands. At cleavages, chromosomes with asymmetrical hydroxymethylation of sister chromatids appear. They decrease in number during cleavages, whereas totally non-hydroxymethylated chromosomes become numerous.
Thus, in the zygotic genome, 5hmC is distributed selectively and its pattern is determined by both parental origin of chromosomes and type of chromosome bands - R, G or C. At cleavages, chromosome hydroxymethylation pattern is dynamically changed due to passive and non-selective overall loss of 5hmC, which coincides with that of 5mC.
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J17.09

Investigation of the methylation status of extracellular matrix proteins-encoding genes in normal tissue and breast cancer

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Extracellular matrix proteins play significant role in tumor development. We evaluated methylation status for 12 laminin-encoding genes (*LAMA1, LAMA2, LAMA3A, LAMA3B, LAMA4, LAMA5, LAMB1, LAMB2, LAMB3, LAMC1, LAMC2, LAMC3*), 8 genes of integrins (*ITGA1, ITGA2, ITGA3, ITGA4, ITGA6, ITGA7, ITGA9, ITGB1*), 2 nidogen genes (*NID1,NID2*), 2 genes of the cadherin family (*CDH2,CDH3*), the dystroglycan gene *DAG1*, 9 matrix metalloproteinases-encoding genes (*MMP2, MMP14, MMP15, MMP16, MMP17, MMP21, MMP23B, MMP24, MMP28*) and the metalloproteinase inhibitor 3 gene *TIMP3* in 194 samples of breast cancer, 194 paired adjacent nonmalignant samples and 6 samples of normal mammary gland from autopsy.
Promoters of 15 genes, *LAMA1, LAMA2, LAMB1, ITGA1, ITGA4, ITGA7, ITGA9, NID1, NID2, CDH2, CDH3, MMP2, MMP23B, MMP24, MMP28* have demonstrated abnormal methylation in 1,5% to 38% samples of breast cancer and/or adjacent tissues.
Promoters of the *LAMA3A, LAMB2, LAMB3, LAMC2, MMP14, MMP21* genes

were constitutively methylated in breast tissues.
Extracellular matrix proteins genes abnormally methylated in breast cancer

Gene symbol	Methylation in breast cancer and/or adjacent nonmalignant samples (%)	Methylation in normal mammary gland from autopsy (%)
LAMA1	29,4 (50/170)	0
LAMA2	25,8 (48/186)	0
LAMB1	28,5 (51/179)	0
ITGA1	15,2 (29/190)	0
ITGA4	29,8 (58/194)	0
ITGA7	3,1 (6/190)	0
ITGA9	38,9 (74/190)	0
NID1	36,2 (63/174)	0
NID2	38,7 (71/183)	0
CDH2	7,7 (14/181)	0
CDH3	33,3 (61/183)	0
MMP2	3,2 (2/63)	0
MMP23B	14,3 (9/63)	0
MMP24	9,3 (5/54)	0
MMP28	1,5 (1/63)	0

J17.10
Exosome-associated miR-146a as a potential lupus nephritis biomarker in urine

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Introduction: Changes in urinary miRNAs have been reported in several renal diseases, having a strong potential to be biomarkers of renal injury. We investigated if miRNAs in urine are concentrated in exosomes and whether a glomerular disease as lupus nephritis (LN) modifies the distribution pattern.
Methods: We used urine of patients with systemic lupus erythematosus (SLE) (6 active, 10 inactive and 12 absence of LN) and healthy controls (n=12), quantifying miRNAs by RT-qPCR in cell-free urine (CFU), exosome-depleted supernatant (Sn) and exosome pellet (Exo). Selected miRNA were: ubiquitously detected in urine (miR-302d, miR-335*), kidney damage biomarker (miR-200c) and altered in glomeruli of LN (miR-146a). Vesicles were characterized by TEM, western blot and NanoSight.
Results: In active LN, all miRNAs were significantly higher in Exo compared to Sn, especially miR-146a (57-fold change, $p<0.01$). Furthermore, comparing miRNAs of each urinary fraction among pathological groups with controls, we observed that miRNAs of Exo in active LN were significantly increased, being miR-146a the most augmented (103-fold change, $p<0.001$). Inactive LN only showed a significant increase for miR-146a ($p<0.05$). Finally, urinary exosomal miRNA-146a had the highest diagnostic role of active LN compared to SLE without LN (AUC 0.960, $p<0.01$), and between active and inactive LN (AUC 0.867, $p<0.05$). Moreover, logistic regression was performed for exosomal miR-146a (OR 24.00, $p<0.05$).
Conclusions: This study confirms that urinary miRNAs are enriched in exosome-containing pellet. In the presence of active LN, the quantity of miRNAs was increased, especially in isolated exosomes. MiR-146a showed high diagnostic accuracy of active LN, underlining the importance of exosome-associated miRNAs as renal disease markers.

J17.11
Microparticles as vectors of microRNAs in HIV-1 infection

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HIV (Human Immunodeficiency Virus) infection induces a state of immune activation associated with chronic inflammation. Chronic inflammation contributes to the pathogenesis of many chronic medical illnesses. Membrane-derived microparticles (MP), released from cell surface, are implicated in cell to cell communication. Recent data evidenced the presence of miRNAs in MP that could mediate exchange of biological information.
We hypothesized that MP produced by CD4 T lymphocytes, which are target cells for HIV infection, are vehicles for miRNAs and could transfer them in receiver cells to alter the expression of numerous genes, in particular those related to inflammation. The aim of the study is to demonstrate the role of miRNAs transfer by MP in pathophysiology during HIV infection.
We analyzed the expression of 375 miRNAs in CD4 T cells, in CD4 T cells-derived MP and in circulating MP from HIV-1 patients receiving highly active antiretroviral therapy, from never-treated (naive) HIV-1 patients and from seronegative age- and sex-matched control individuals. This analysis showed that several miRNAs significantly differentiated HIV-1 patients from uninfected individuals. We identified 2 overexpressed miRNAs in both CD4 T lymphocytes, in CD4 T cells-derived MP and in circulating MP from naive HIV-1 patients compared to controls, which are of interest because they were described as downregulators of NF-kB pathway.
This study provides an opportunity to better understand mechanisms of inflammation in HIV infection. MP, and miRNAs they contain, could then be considered as therapeutic targets and MP production could be regulated in new therapeutic strategies.
This work was supported by grants from ANRS (2011 and 2012).

J17.12
Early Detection of Preeclampsia using Circulating small non coding RNA

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Preeclampsia is one of the most dangerous pregnancy complications, and the leading cause of maternal and perinatal mortality and morbidity; yet its cause remains unclear. Current theory of preeclampsia pathogenesis states that it begins with poor placentation in the first trimester of pregnancy. Thus, although clinical symptoms are late, early detection of preeclampsia can be feasible at the first trimester. Recent findings suggest that circulating small non-coding RNAs (ncRNAs) in the mother's blood may be effective markers for early diagnosis of preeclampsia, however as of yet such ncRNAs were identified only in late stages of the pregnancy and have not been implemented in clinical practice. Furthermore, mapping ncRNA expression at an early stage of the disease might shed light on the possible mechanisms involved in the disease etiology.
We have compared small ncRNAs in plasma of first trimester pregnant women with and without preeclampsia. To this end, we have performed small ncRNAs Next Generation Sequencing (NGS) of preeclampsia and control samples, and identified several transcripts significantly differentially expressed between the two sets. We further utilized the list of these transcripts and created a pipeline for supervised classification of preeclampsia versus control samples. The classification pipeline obtained high accuracy in preeclampsia samples prediction. Furthermore, applying the procedure on two different ethnic groups resulted in high accuracy values as well, which demonstrates our method's generalization capability.
Our findings lay the foundation for an early non-invasive diagnostic tool of preeclampsia based on circulating small ncRNAs, in order to lower the life-threatening risk for the mother and fetus.

J17.13
Beckwith-Wiedemann and Russell-Silver syndromes: Evidence of multilocus methylation defects.

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The imprinted 11p15 region is crucial for the control of foetal growth and loss of imprinting at this locus is implicated in two clinically opposite disorders: Beckwith Wiedemann syndrome (BWS) and Russell-Silver syndrome (RSS). In order to investigate multilocus methylation defects in a series of 33 patients referred for BWS and RSS, we studied the methylation status of 4 maternally (6q24, 7q32, 7p12 and 11p15) and 2 paternally (11p15 and 14q32) methylated loci.Seven out of 14 BWS patients harbored maternal ICR2 hypomethylation and one BWS patient had both ICR1 and ICR2 defects which indicated paternal 11p15.5 uniparental disomy. Among the 19 suspected RSS patients, the diagnosis was confirmed in only one patient with a typical RSS phenotype with severe Intrauterine growth retradation, facial dysmorphisms and psychomotor retardation, and who showed maternal 11p15.5 duplication. Multilocus methylation defects concerns 2 patients out of 33. The first patient referred for BWS carried both maternal ICR2 hypomethylation and 7q32 hypomethylation, thusboth BWS and RSS diagnosis. He presented a BWS phenotype with macrosomia, macroglossia, facial

dysmorphisms and hypothyroidism and no obvious RSS clinical features. The second patient referred for BWS with macrosomia, macroglossia and hypoglycemia, surprisingly carried 7p12 and 6q24 hypomethylation, thus combining RSS and transient neonatal diabetes mellitus diagnoses.Although our series of RSS and BWS patients had Loss of methylation at loci involved in other human diseases, they did not generally show evidence of other clinical disorders. This is consistent with a phenotypic (epi)dominance of the 11p15 region Loss of imprinting in our series of patients.

J17.14
Differential methylation profiles of ten patients disclose the glutamate pathways association with attention deficit hyperactivity disorder

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Attention deficit hyperactivity disorder (ADHD) is one of the most common childhood brain disorders and implying strong familial, genetic and environmental risk factors. Despite the prevalence of ADHD, molecular markers for the diagnosis of ADHD have not yet been identified. DNA methylation is an important epigenetic mechanism associated with silencing of genes in CpG islands. In this sense, the investigation of methylation profile in patients with ADHD could reveal important aspects of the disease.
We performed the Genome-wide methylation profile using DNA extracted from blood lymphocytes of 10 patients with ADHD (ages 08-15) with Illumina Infinium HumanMethylation450 BeadChip. Array data were pre-processed using GenomeStudio software and full analysis was performed using specific packages within the environment R. Ingenuity Pathway Analysis was used to identify canonical pathways of the genes that were enriched in the list of CpG loci with significantly different methylation in ADHD patients.
Our analyzes showed significantly methylated probes involved in several networks, including Glutamate Biosynthesis and Glutamate Degradation X pathway, pointing out the relevance of glutamate, which is closely associated to the Central Nervous System and could be modified by epigenetic factors.
Thus, profile the tissue-specific DNA methylation patterns will provide novel insights into pathogenic mechanisms of ADHD, as well as, help in future epigenetic therapies.
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J18.01
Association between G-2548A leptin gene polymorphism and age at menarche

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Background: Early menarche is a risk factor for teenage depression, insulin resistance, and breast cancer in adulthood. Among the various social, economic, biological, and environmental factors implicated in the secular decline in age at menarche, There is growing evidence that childhood obesity is an important determinant of early menarche. Obese children have high leptin levels, and these may play a role in their earlier onset of puberty. Leptin is one of many proteins important to obesity that secreted by adipocytes in proportion to adipocyte tissue mass. Lep-2548G/A polymorphism in the promoter region of leptin gene, that has previously been shown to correlate with variations in serum leptin levels and degree of obesity. The aim of this study was to evaluate the impact of leptin G-2548A gene polymorphism on the age at menarche in a sample of the Iranian population.
Material and Methods: This study was done on 374 Iranian women from February 2012 to February 2013. The leptin genotypes were determined using PCR-RFLP method and age at menarche was obtained by questionnaires. Data analysis was performed by SPSS version 18.
Result: women carrying the AA genotype had a significantly younger age at menarche (12.47 years) than women with the AG (12.94 years) and GG (13.47 years) genotypes. Also, we found that the AA genotype frequency in women with age at menarche <13 years was higher than in women with age at menarche ≥13 years (OR:3.4, 95%CI:1.7-6.7, P:0.001).
Conclusion: The G-2548A leptin gene polymorphism has an important role in the onset of menarche.

J18.02
Candidate genes sequence variants in patients with congenital heart defects

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Congenital heart disease (CHD) affects the structure and function of the heart and is the most common major malformations and the largest proportion of mortality caused by birth defects in pediatric age. The current work engaged in identifying genomic variants and its effects in genes linked to sporadic CHD cases. To achieve this aim, DNA was extracted and purified, then followed by standard sequencing immediately. We used the bioinformatics tools to predict the effects of an outcome variants and was selected which have high predictor value to be under the functional genetic variant evaluation. The results revealed that many reported and unreported genetic variations were founded in GATA4, TBX5, Nkx2.5 and HAND2 genes, most of these variations were founded in GATA4 and TBX5 genes that underwent the functional analysis.
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J18.03
Candidate gene analysis of permanent tooth agenesis

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Permanent tooth agenesis is a common human anomaly of complex etiology that affects approximately 7 %, based on population based study (OMIM: #106600, #604625). It may occur independently, or as one feature of a syndrome. The non-syndromic forms may be sporadic or familial with prevalence varying by tooth type. According to severity, selective tooth agenesis can be divided into hypodontia, and oligodontia. Hypodontia is generally defined as agenesis with absence of less than six teeth, and oligodontia is a condition in which six or more teeth are missing. Mutations in MSX1 and PAX9 are associated with specific forms of tooth agenesis. In the dental mesenchyme, MSX1 is detected in the dental epithelia during the tooth development, and PAX9 is expressed in the neural-crest-derived mesenchyme of the maxillary and mandibular arches. In addition, several lines of observation have indicated that mutations in isolated tooth agenesis were detected in AXIN2, EDA, and WNT10A. Here we report mutation analyses of four candidate genes (MSX1, PAX9, WNT10A, AXIN2) in 72 probands (32 hypodontia and 40 oligodontia) in Japanese. A mean number of missing teeth was 7.1. We identified 17 mutations. Together with our previous reports, we have identified seven mutations in MSX1, two in PAX9, six in AXIN2, and two in WNT10A. MSX1 and PAX9 mutations were identified in 32.2%, and 54.5% of cases, respectively. Our findings on the intra oral distribution of agenesis of permanent teeth in children may help us better understand the etiology of agenesis.

J18.04
Epidemiology of Prothrombin G20210A Polymorphism in the Southern Iran, 2014

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Background: There are many genetic and non-hereditary risk factors that are known to cause venous thromboembolic (VTE) disorders. One of these is the Prothrombin G20210A mutation. Moreover, the association of PT G20210A polymorphisms with cancer has been reported. The present study was designed to determine the frequency of PT G20210A polymorphism in Southern Iran.
Methods: 140 healthy women were selected from Namazi Hospital in Shiraz city. A total of 5 ml of peripheral blood was taken from individuals then Genomic DNA was extracted using BioRon blood DNA kit. The ARMS-PCR method was used for the detection of PT G20210A single nucleotide polymorphism in each subject.
Results: The prevalence of G/G, G/A, and A/A genotypes was found to be 97.9%, 2.1%, and 0%, respectively.
Conclusion: Results of the present study might be important in understanding the distribution of PT G20210A polymorphism in the Iran population. Minor allele frequency (MAF) in this population was 1.1%.

J18.05

To compare prevalence of endometriosis associated common polymorphism in Shiraz endometriosis clinics patients with control groups

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Background:Endometriosis is a common gynecologic disorder defined as ectopic presence of endometrial tissue in extra-uterine sites. Higher stages of endometriosis are associated with infertility and higher risk of malignancy. Identification of responsible gene alterations and mutations can improve therapeutic approaches. In the present study we investigated association of ARID1A and eNOS polymorphisms with endometriosis and staging of the disease.
Materials and methods:100 women with laparoscopy confirmed diagnosis of endometriosis were included and compared with 100 non-endometriosis women as the control group, regarding eNOS and ARID1A polymorphisms.
Results:Significantly higher prevalence of non-CC genotype for ARID1A Gln920Ter polymorphism and non-GG genotype for G894T polymorphism of eNOS gene was detected in endometriosis group. There was no significant relationship between these polymorphisms and staging of endometriosis.
Conclusion and discussion:The results of this study suggest that mentioned polymorphisms of ARID1A and eNOS genes may play role is development of endometriosis rather than staging and progression of the disease.

J18.06

GENETIC STUDY MEFV GENE OF PERIODIC DISEASE : ABOUT 12 PATIENTS MAROCAINS

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Familial Mediterranean Fever (FMF) , also called periodic disease is a hereditary inflammatory disease. It is of particular geographic and ethnic distribution. Indeed, it is mostly observed in populations from around the Mediterranean (Arabs, Sephardi Jews , Turks and Armenians) . It is a genetic disorder autosomal recessive , due to mutations in the MEFV gene on chromosome 16. More than two hundred variants have been described, but only twelve mutations (E148Q , M680I , M680L , T681I , I692del , M694I , M694V , M694del , K695R , V726A , A744S , R761H) are frequently associated with most cases of FMF.
12 patients were recruited to the Medical Genetics Unit of the University Hospital Hassan II FES , seven women and five men. Genomic DNA was extracted from peripheral blood using the standard protocol of „ salting out „ and the MEFV gene was investigated by PCR- sequencing specific research of the most common mutations in exon 10 .
Molecular analysis conducted in patients showed that one patient carried the M694I mutation in the homozygous state . Eleven patients did not have the desired mutations , with a very suggestive clinical disease and a good response to the test treatment, which does not exclude the presence of other not yet sought mutations in other exons. Thus, it would be interesting to look for other known MEFV gene mutations involved in this disease in order to provide adequate genetic counseling to all patients .

J18.07

Trends in the prenatal diagnostics of the Down syndrome in the Czech Republic: What is the association with the maternal age?

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Introduction: Down syndrome is the most common autosomal aneuploidy syndrome. Total incidence (including prenatally and postnatally diagnosed cases) of the Down syndrome in the Czech Republic is increasing during the last two decades. The two mainly discussed causal factors are the improvement of the prenatal screening/diagnostics methods and the eminent increase of the average maternal age.
Methods: Retrospective population-based analysis of the prenatally diagnosed cases of the Down syndrome in the Czech Republic (1998 - 2011 time period). Total number of 2 110 cases were analyzed.
Results: The relative number of the prenatally diagnosed cases of the Down syndrome has increased from 10.82 per 10 000 (in 1998) to 20.24 per 10 000 (in 2011), while the incidence in births has decreased from 6.41 (in 1998) to 3.50 (in 2011, relative numbers per 10 000 are given). The average gestation week at the time of prenatal diagnosis of the Down syndrome has decreased from 19.98 (in 1998) to 15.55 (in 2011). In 1998 the majority of Down syndrome cases were diagnosed in 20-22th week of gestation, while in 2011 the diagnosis was made mainly in 12-13th week of gestation.
Conclusion: We have found significant changes in the prenatal diagnostics of the Down syndrome. While in 1998-2003 time period the Down syndrome was diagnosed early in elder women (women aged 35 years or over - who chose karyotyping because of advanced maternal age indication) we have not found this connection in the 2004-2011 time period. This change represents the increasing role of the first trimester prenatal screening in the Czech Republic.

J18.08

DNA microarray for the diagnostics of monogenic hereditary disease in Yakuts.

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Yakut people belongs to the monogenic population with ethnicity specific form of pathology, around ten hereditary diseases with the frequency significantly more higher than in other populations in the world. The molecular genetics research showed the presence of major mutations specific and often found among people of Yakut origin. One of the major problems for now is to do a screening for the found mutations, diagnostics of the diseases and to organize preventive care.
In collaboration with colleagues from „Alcor Bio" Co Ltd., Saint Petersburg, Russia there was established a testing system based on DNA microarray for the diagnostics of five high frequency diseases among Yakuts. This method has a lot of advantages and the main one is a possibility to screen several mutations at once comparing to routine methods such as PCR and restriction. It makes the diagnostics simple, fast and money saving.
There were five diseases with point mutations chosen:
1. Enzymopathic methemoglobinemia (OMIM 250800, Pro269Leu mutation in DIA1 gene)
2. 3 M syndrome (OMIM 273750, 4582insT in Cul7 gene).
3. SOPH syndrome (OMIM 614800, G5741 A in NAG gene).
4. Nonsyndromic hearing loss 1A type (OMIM220290, IVS1+1G>A in GJB2 gene)
5. Tyrosinemia type1 (OMIM 276700 1090G>C mutation in FAH gene).
Since the microarray technology is well known and rapidly developing in the world, the results of specific research of Yakut population itself using these biochips rather than then biochip establishment will have the significance. Until now the setting up the methods and optimization of procedures is carring on.
The research is supported by Federal special purpose program 2014-2016 in Russia

J18.09

Genome-wide association study of elite strength athlete status in Russians

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Humans vary in their ability to achieve success in strength sports. The aim of the study was to identify SNPs associated with strength athlete status in Russians using a GWAS approach. The study involved 483 Russian athletes (49 strength athletes, 103 endurance athletes and 331 athletes from other sports with strength component: 89 sprinters, 38 strength/speed athletes, 64 wrestlers, 42 rugby players, 98 rowers/kayakers/canoeers) and 173 controls. HumanOmni1-Quad BeadChips (Illumina Inc, USA) were used for genotyping of 1,140,419 SNPs in Russian athletes and controls. Initially, 43 SNPs associated with elite strength athlete status (when compared with controls) with P<10-5 were identified, but none of them reached genome-wide significance level. Adding three criteria, i) an increase of the frequency of effect allele with increase of the level of achievement of strength athletes; ii) significant differences in allelic frequencies between strength athletes and endurance athletes; iii) at least one replication of association between effect alleles and predisposition to other sports with strength component, resulted in remaining eight SNPs (SUCLA2 rs10397 A, MED4 rs7337521 A, GPC5 rs852918 A, GABRR1 rs282114 A, CACNG1 rs1799938 A, ARHGEF28 rs17664695 G, WAPAL rs4934207 G, MPRIP rs6502557 A alleles) with P va-

lue from 9.1x10-5 to 3.1x10-6. These SNPs are located in the genes involved in the regulation of ATP production (SUCLA2), transcription of DNA (MED4), cell division and growth (GPC5, ARHGEF28), neurotransmission (GABRR1), muscle contraction (CACNG1, MPRIP) and DNA repair (WAPAL). Our results suggest that many genetic variants may influence strength performance but additional replication studies are warranted.

J18.10
Genetic diversity of human populations in genes associated with immunity-dependant diseases

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The aim of this investigation was to study the genetic diversity in genes associated with allergic and autoimmune diseases and with regulation level of cytokines and immunoglobulin E levels in North Eurasia. 26 population groups from Northern Eurasia were analyzed. 27 markers in 24 genes were selected to perform statistical analyses of data collected in our populations of Northern Eurasia as well as in 31 populations from HapMap, 1000 Genomes and HGDP projects. The frequencies of alleles and genotypes within the populations demonstrated wide variability. Strong Spearman correlations of allele frequencies with absolute longitude and year range of average temperatures were revealed for the majority of loci. Significant correlations between average expected heterozygosis in 27 loci and absolute latitude, temperature of the coldest month, range of average temperatures and average annual level of precipitations were showed. The estimation of population genetic relations by principal components analysis has revealed the clustering of populations according to their geographic location. Participation of investigated genes in Jak-STAT signaling pathway, in interaction between cytokines and their receptors, in differentiation of lymphocytes by Th1/Th2-pathway have been identified using DAVID on-line recourse. Thus, the obtained data indicate that genetic diversity in genes associated with immunity-dependant diseases was probably shaped by adaptation to new environment during human dispersal in North Eurasia.

J18.11
Analysis of the Y-chromosome in the Volga-Ural region populations from Russia

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We analyzed a sample of the Volga-Ural region, including 462 individuals from 8 populations: Udmurts, Komi, Mordvinians, Mari, Besermyans, Chuvashes, Tatars, Bashkirs. We have shown that the major proportion of Y-chromosome haplogroups in the studied populations accounted for the four branches (R1b-M269, R1a-M198, N1c1-Tat and N1c2-P43), which together make up from 51% to 100% of the patrilineal genetic diversity in the studied region.

We have shown that West Asian and Central Asian Y-chromosome haplogroup R1a-Z2125 in the Volga-Ural region occurs with the greatest frequency in Bashkirs (31%), which is the dominant subgroup of haplogroup R1a-M198 in this population despite the fact that in other populations Eastern European R1a-M558 and R1a-M458 are the dominant lines. This fact indicates that different haplogroup R1a-M198 lines in the populations of the Volga-Ural region have different sources.

The Eastern European influence in the population can be also seen in Tatars from Tuimasinsky district of Bashlortostan in which typical for Central Europe haplogroup R1b-M405 is the predominant line of the haplogroup R1b-M343. According to the PCA analysis based on the Y-chromosome haplogroups distribution, Bashkirs show the greatest separation from other populations of the region. The reason is the presence with the high frequency of Asian lineages in their gene pool.

J18.12
Statistical analysis for parentage test and mutation rate determination in Thai database

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Introduction: The analysis of DNA results for parentage test is expressed based on a mathematical as the combined paternity index (CPI) calculation. In case of mismatches between the father or/and mother and the child alleles, there is a chance of mutation in some loci.

Materials and Methods: Using DNA typing to determine the relationship between parents and child from 1,011 unrelated families. The DNA samples were amplified using commercial kits for 9 and 15 STR loci to establish kinship analysis of trio case from 324 families and then duo cases between fathers-child and mothers-child in 305 and 382 families, respectively.

Results: In trio cases the CPI of 376 values were 420.07 to 359,558,681.35. In duo cases of father - child, the CPI of 423 values were 228.71 to 38,900,000,000 and mother - child of 543 values were 70.45 to 1,730,000,000. In 1,377 parent/child allelic transfers, 35 isolated STR mismatches were observed. The increase or decrease of short tandem repeats (n=34) and a single base change (n=1) were detected. The mutation rate was 0 to 5.17 x 10^{-3} per locus per gamete per generation.

Conclusion: This report proposed a model for analysis of the variations in the distribution for CPI value in parentage test. The minimal value of CPI may be use to determine the cut off for the relationship criteria. In addition, the mutation rate may help for more reliable in calculation of CPI in mismatched case.

J18.13
The molecular spectrum of α- globin gene mutations in suspected of alpha-thalassemia carriers in south of Iran

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In this study, 895 individuals with low hematological values, normal Hb A2 who were not affected with β- thalassemia or iron deficiency, were investigated for the presence of α- thalassemia mutations. α-thalassemia mutations were characterized by gap polymerase chain reaction (gap-PCR), MLPA, and sequencing for both α genes. The most common deletion was -α(3.7) with a frequency of 57.9% and 12.1% for -α3.7 /αα and -α3.7/-α3.7 respectively, followed by α CD19(-G) α/αα (2.6%), αpoly A6α/αα (2.3%), -α4.2 /αα (1.91%), --Med/ αα (1.7%), α IVS donor site α/αα (1.2%),--20.5/ αα (0.8%), α Constant springα/αα (0.7%), αpoly A4α/αα (0.3%) and, etc. The results of this study will be useful for genetic counseling and the prevention of HbH disease in Khouzestan Province, Iran

J18.14
Analyze of SNP rs9939609 in FTO gene of obese males in Iraqi population

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Abstract

The present study conducted on 120 and 50 obese and healthy males respectively, their age ranged from 20-50 years. The patients were divided into 3 groups based on Body Mass Index (BMI) and Central Obesity (CO); it has noticed that there is a significant relation between both indexes. DNA from blood of obese and healthy was isolated and SNPrs9939609 was then amplified by PCR using appropriate primers, genotype was identified using *Sac*I. Parameters TG, TC, HDL, LDL, VLDL and FTO enzyme level have been investigated. The results showed that there are significant differences P≤0.05 for AA genotype with all parameters whereas TA genotype showed significant differences with most of parameters in revers to TT genotype which has showed no significant differences with most of parameters. The percentage of AA, TA, TT alleles were 27.72%, 49.86%, 22.42% respectively, also an elevated of TT genotype was observed in healthy compare with obese. On the other hand the percentage of T and A allele frequency were 52.65% and 47.35% respectively.an elevated in serum FTO enzyme was observed in obese.

Conclusion: the presence of A risk allele in the Iraqi population is the cause in the incidence of obesity which reflected its impact on the BMI and CO through the turbulence in lipid profile and FTO enzymes value.

Table 1: Effect of SNP rs9939609 genotypes on investigated parameters

p-values			Parameter	No.
AA	TA	TT		
0.037	0.002	0.204	BMI Kg/m^2	1.
0.015	0.082	0.322	CO. cm	2.
0.001	0.001	0.599	TG mg/dl	3.
0.001	0.001	0.495	TC mg/dl	4.
0.016	0.843	0.001	HDL mg/dl	5.
0.001	0.029	0.005	LDL mg/dl	6.
0.001	0.031	0.004	VLDL mg/dl	7.
0.011	0.005	0.757	enzyme *FTO*	8.

Figure 1: BMI and CO in obese and healthy

J18.15

UGT1A1*16 and UGT1A1*28 alleles in 17 consanguineous Tunisian families from SIDI BOUZID and GAFSA

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We report a study of 17 Tunisian families with suspicion of Crigler-Najjar syndrome type I (CNS1). Molecular analysis was carried out using PCR and direct sequencing of the TATA box and exon 3 of UGT1A1 gene and then the remaining four exons of the gene. The 17 families were exclusively native of Sidi Bouzid and Gafsa, from specific delegations of these two towns: Bir El Hafey, Meknassy, Regueb, Sidi Bouzid, Gafsa and Mdhilla. 16 among 17 index cases were homozygous for the c.1070A>G mutation (UGT1A1*16) and for the TA insertion in the TATA box of the UGT1A1 gene promoter, resulting in TA7/TA7 genotype (2 UGT1A1*28 alleles or 2 A(TA7)TAA alleles or 7/7 homozygous genotype) or TA8/TA8 genotype particularly for some patients from Gafsa. Mutations were located on the same chromosome, as the analysis of the index patient reveals. 15 parents and related families members were heterozygous carriers of the TA insertion and the linked c.1070A>G mutation. Only 2 partners from Gafsa had TA7/TA8 genotype [(TA)7,8] with a child who was 8/8 homozygous.
These results indicated that CNS1 in Tunisia seems be associated with two founder effect related to the UGT1A1*16 (Q357R) mutation linked to TA7 (UGT1A1*28) or to TA8 alleles within two regions of the Western Centre. Consanguineous marriages, had limited the diffusion of these mutations for some times. But, at present, Tunisian's sanitary authorities have to set up a national strategy to offer genetic counselling and molecular preconceptional and prenatal diagnosis to prevent specially CNS1 new cases.

J18.16

Genome-wide association study reveals seven genetic markers associated with maximal oxygen consumption rate in elite Russian endurance athletes

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The aim of the study was to identify SNPs associated with elite endurance performance (maximal oxygen consumption rate, VO2max) and endurance athlete status in Russians using a GWAS approach, genotype-phenotype and case-control designs. The study involved 542 international-level athletes (103 long distance athletes, 120 middle distance athletes, 319 power athletes) and 173 controls. VO2max was measured in 71 endurance (41 males and 30 females; 26 long distance and 45 middle distance) athletes. HumanOmni1-Quad BeadChips (Illumina Inc, USA) were used for genotyping of 1,140,419 SNPs in Russian athletes and controls. No genome-wide significant results were observed. However, when we used the criteria that any SNP should be independently associated with VO2max in male and female athletes separately (with P<10-3 adjusted for sex), and the frequency of the endurance-related allele should be over-represented in endurance athletes in comparison with controls and power athletes, we identified seven alleles (ZNF429 rs1984771 G, FMNL2 rs12693407 G, ACOXL rs13027870 G, NFIA-AS2 rs1572312 C, ITPR1 rs2131458 A, GALM rs3821023 A, NATD1 rs732928 G) with suggestive significance in the determination of endurance performance. These SNPs are located in the genes involved in the regulation of lipid (ACOXL) and carbohydrate (GALM) metabolism, erythropoiesis (NFIA-AS2), morphogenesis and cytokinesis (FMNL2), intracellular Ca2+ signaling (ITPR1) and other processes (ZNF429, NATD1). Our results suggest that many genetic variants of small effect size may influence human physical performance.

J18.17

Gene expression profile at different age periods among residents of Bashkortostan

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Functional activity of transcriptome is decrease with age generally. This leads to age-related changes of organs and tissues. The aim of the present study was to evaluate the role of expression level of some genes for different age stages, in particular for senile age and age of longevity. For this purpose was performed gene expression analysis in groups of senile (75-89 years old) and long-lived (90-100 years old) persons in comparison with middle-age persons (25-39 years old). Total RNA was isolated from peripheral blood leukocytes of 60 healthy subjects, man and women, residents of Bashkortostan Republic, Russia. Quantitative real-time PCR was performed using RT2 SYBR Green/Fluorescein qPCR Master Mix and RT2 qPCR primers for IL6, JAK3, STAT5, TNFA, LAMA2, ACE, TEAD and COL13A1 genes. It was found tendencies to increase the expression level of mRNA gene IL6 in senile group compared to middle-age persons (2.14-fold). Among long-lived persons reduced the expression level of TNFA (3.28-fold) and LAMA2 (5.87-fold) genes. Transcription activity of LAMA2 gene in group of long-lived persons in 10.6 fold lower compared to the senile group (p=0.05). These data suggest a positive role of IL6 cytokine for senile age and negative character of TNFA and LAMA2 genes in the reaching of longevity. So, aged persons had altered cytokines and LAMA2 genes expression profile.
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J18.18

High proportion of W1282X mutation in Karachai CF patients

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Cystic fibrosis (CF; OMIM #219700) is a common autosomal recessive disease. The spectrum and frequency of CFTR mutations significantly vary in different populations and ethnic groups. The Karachai - Turkic-speaking people of the North Caucasus (the indigenous ethnic group of the Republic of Karachay-Cherkessia (Russia), constituting about 40% of the Republic population (194 thousand)), live compactly in 4 districts, of which three were included in the genetic-epidemiological study. The cystic fibrosis prevalence was 1:2878 children in Ust-Jegutinsk, 1:2875 children - in Karachaevsk, 1:4630 children - in Malokarachaevskiy district.
Molecular genetic analysis for 29 CFTR mutations (shared up to 75-80% of mutant alleles in Russian population) was performed in 9 unrelated Karachai CF patients. A high proportion of W1282X mutation was revealed (16 of 18 mutant alleles, 89%): seven patients - homozygous for W1282X mutation, two - compound heterozygous (second allele has been not identified yet).
In 105 healthy Karachai the analysis of the 13 CFTR mutations (CFTRdele2,3(21kb), F508del, I507del, 1677delTA, 2184insA, 2143delT, 2183AA>G, 2184delA, 394delTT, 3821delT, L138ins; E92K; W1282X) identified one 1677delTA mutation carrier and one W1282X mutation carrier.
Thus, neither among the CF patients nor among the healthy Karachai the most common CFTR mutation (F508del) was detected. The most frequent mutation among CF patients was W1282X. Its frequency in healthy Karachai was about 0,005. Until now, the origin and spread of W1282X mutation was associated with the settlement of the Ashkenazi Jews. Further investigation of linked DNA markers could clear up the origin of W1282X mutation in Karachai.
The research was partially supported by grants RFFR 14-04-00525; 15-04-01859.

J18.19

Different GJB2 gene contribution to deafness in ethnically matched patients from two regions of South Siberia

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Mutations in gene GJB2 (MIM 121011, 13q11-q12) which encodes gap junction protein connexin-26 (Cx26) are one of the main causes of nonsyndromic deafness. Pathogenic contribution and mutational spectrum of GJB2 are known to depend on population ethnic origin. The main objective of this study is a comparative estimation of the GJB2 pathogenic contribution to deafness in populations of two South Siberian regions (the Altai Republic, the Tyva Republic). The results were obtained on 364 ethnically matched patients (93 Altaians, 192 Tuvinians, 40 Russians, 13 Kazakh, and 26 pa-

tients of mixed ethnicity) with congenital or early onset hearing loss. The GJB2 (Cx26) pathogenic contribution to hearing impairment was defined as proportion of so called Cx26-positive patients with two homozygous or compound heterozygous recessive (or one dominant) GJB2 mutations. Mutation screening of GJB2 was performed by Sanger sequencing of GJB2 protein-coding region (exon 2), exon-intron 1 region and flanking sequences that revealed full set of GJB2 pathogenic mutations and polymorphisms in examined patients. We found similar proportion of Cx26-positive patients among Altaians (15.1%) and Tuvinians (18.8%) belonging to two indigenous peoples of South Siberia that substantially lower than in Russians (47.5%) living in studied regions. Equal GJB2 contribution in deafness (23.1%) was found in Kazakh and in group of patients of mixed ethnicity. Three GJB2 mutations c.235delC, p.W172C, and IVS1+1G>A are common in Altaians and Tuvinians, while mutation c.35delG is predominant in Russian and Kazakh patients. Study was supported by the State research project VI.58.1.1 and the RFBR grants #15-04-04860_a, #14-04-90010_Bel_a.

J18.20
The difference in genomic profiles between endurance and power athletes

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In terms of physiology and metabolism, power and endurance are located at the opposite extremes of a muscle performance continuum. The aim of the study was therefore to identify the difference in genomic profiles between endurance and power athletes by using a GWAS approach. The study involved 542 Russian athletes (319 power athletes, 223 endurance athletes) and 173 controls. HumanOmni1-Quad BeadChips (Illumina Inc, USA) were used for genotyping of 1,140,419 SNPs in all cohorts. In addition, endurance performance (expressed as a maximal oxygen consumption rate, VO2max) was measured in 71 endurance athletes. At the first stage, by comparing genetic profiles of two groups of elite athletes only (171 elite power and 56 elite endurance athletes), we identified 13 SNPs with suggestive significance (P values from 10-5 to 10-6). We then compared allelic frequencies of discovered SNPs between each group of athletes and controls and performed regression analysis to reveal association with VO2max of endurance athletes. These analyses resulted in remaining 3 SNPs (effect alleles: CLSTN2 rs2194938 C, FOCAD rs17759424 C, TPK1 rs10275875 G) associated with power athlete status and 5 SNPs (CLSTN2 rs2194938 A, TPK1 rs10275875 A, ITPR1 rs1038639 A, NALCN-AS1 rs4772341 A, SPOCK1 rs1051854 A) with endurance athletes status (based on case-control study and correlation with VO2max). These SNPs are located in the genes involved in the regulation of neuronal excitability (CLSTN2, NALCN-AS1), cell growth (FOCAD), vitamin B1 metabolism (TPK1), muscle contraction (ITPR1) and protein metabolism (SPOCK1).

J18.21
Between seas and steppes: the genetic legacy of ancient Greeks and medieval Mongols in population of Crimea peninsula

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Since prehistoric times, the Crimea was an area of intensive migrations. The (incomplete) list of populations migrated there includes Scyphians, ancient Greeks, Romans, Hunns, Gots, Tatars, and Russians. We collected 400 DNA samples from unrelated male volunteers representing two main indigenous populations in Crimea: Crimean Tatars (Steppe, Mountain, and Coastal subethnic groups) and Crimean Greeks (Urum and Romei sub-ethnic groups).
We analyzed Crimean populations by Y-chromosomal markers, full mitochondrial genomes and genome-wide autosomal markers. The results provided by all three genetic systems virtually coincided. We revealed three main features in Crimean gene pools. First, Mountain and Coastal Crimean Tatars and both Crimean Greeks populations are characterized by genetic component predominant in East Mediterranean peoples, especially in Greeks and Turks. Second, Steppe Crimean Tatars carry the contrasting genetic component, which is typical for Turkic populations from Eurasian steppe. Third, all indigenous Crimean populations do not show notable genetic similarity with their closest geographical neighbors - Ukrainians and Russians. We conclude, that present day gene pool of indigenous Crimean populations reflects the distant echo of marine Greek colonization in antique epoch and steppe expansion of Mongol civilization in medieval epoch.
The work was supported by RFBR grants 13-06-00670, 14-06-31331 and by Presidium of RAS Programme.

J18.22
THE Y-CHROMOSOMAL PORTRAIT OF THE INDIGENOUS POPULATIONS FROM THE RUSSIAN FAR EAST

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Populations from the Russian Far East (Amur river basin) have a particular importance for reconstructing the ancient human migrations in Eurasia because the gene pool in this region is supposed to be well preserved since the Neolithic. Using wide panel of Y-chromosomal SNPs we characterized two Manchu-Tungus-speaking ethnic groups - Nanais (N=226) and Ulchis (N=57).
The Nanais (census size about 10,000 persons) have complex population structure. The haplogroups N1c1-M178, C-M130, and C3c-M48 accounts for more than 80% of their gene pool, this feature brings the Nanais together with the Eastern Siberian populations. The less frequent haplogroups O3-M122, O3a2-M201, O1a-M120 indicate gene flow from East Asia. Haplogroups G2a-P15, J2-M172, I-M170, I2a-P37, R1a1-M198 and Q-M242 occur with a frequency of less than 1%.
The Ulchi (about 3,000 persons) dwell the Lower Amur district in Russia. The haplogroup C (C-M130, C3c-M48) comprises 54% of their gene pool; the haplogroup O (O-P186, O1a-M120, O2-P31, O3-M122) accounts for 17%. The rest of the gene pool is formed by I-M170, I2a-P37, J1(xJ1e)-M267, N1c-LLY22, N1c1-M178, and Q-M242 haplogroups.
The surprising peculiarity of the studied populations is the accumulation of haplogroups I-M170* and I2a-P37 which are often though to be European-specific lineages since Mesolithic epoch.
The multidimensional scaling plot revealed a significant genetic similarity of the Nanais and the Ulchis with the Oroch and the Mongols. At the same time the studied Nanai population from Russia is genetically distant from the Nanais sampled in China.
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J18.23
Analysis of potential association between genetic variants in microRNA genes hsa-miR-499 and hsa-miR-196a2 and prostate cancer risk in Serbian population

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Prostate cancer (PCa) is the second most commonly diagnosed cancer among men and also the sixth leading cause of cancer related mortality. Still, genetic factors contributing to PCa susceptibility remain largely unknown. Even though a multitude of evidence supports the involvement of regulatory mechanisms based on activity of microRNAs in prostate carcinogenesis, to date, genetic variants in microRNA genes have been evaluated as potential PCa susceptibility loci in only few populations, none of European origin. Therefore, the aim of this study was to evaluate potential association of rs3746444 in hsa-miR-499 and rs11614913 in hsa-miR-196a2 with PCa risk and progression. The study population included 354 PCa patients and 354 patients with benign prostatic hyperplasia (BPH) from whom blood samples were obtained, as well as 311 healthy controls derived from general population who provided buccal swabs. Genotyping of rs3746444 and rs11614913 was performed by PCR-RFLP and HRMA, respectively. The results did not support the association of analyzed genetic variants with PCa risk. Also, these genetic variant were not found to be associated with the standard prognostic parameters of PCa progression (serum PSA level at diagnosis, Gleason score and clinical stage according to TNM classification system). Nevertheless, rs3746444 minor allele G was shown to be associated with the lower risk of PCa progression (according to D'Amico criteria) under recessive genetic model.

J18.24

A multi-stage genome-wide association study of elite endurance athlete status

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Endurance athlete status is a complex phenotype subject to the influence of both environmental and genetic factors. The aim of the study was to identify SNPs associated with elite endurance athlete status in Russians using a multi-stage GWAS approach (HumanOmni1-Quad BeadChips). The study involved 223 endurance athletes, 67 elite power (sprinters and speed/strength athletes) athletes and 173 controls. VO2max (major indicator of aerobic capacity) was measured in 71 endurance (41 males and 30 females; 26 long distance and 45 middle distance) athletes. Initially, we performed GWAS in 4 subgroups (all and elite long distance athletes, all and elite middle distance athletes) of endurance athletes and controls, and found replications of associations with endurance athlete status in all subgroups for 93 SNPs with P<10-4, but none of them reached genome-wide significance level. Adding three criteria, i) an increase of the frequency of effect allele with increase of the level of achievement of endurance athletes; ii) significant differences in allelic frequencies between 56 elite endurance athletes and 67 elite power athletes (second case-control study); iii) positive correlation of the effect allele with high values of aerobic capacity, resulted in remaining five SNPs (effect alleles: CAMK1D rs11257754 A, CPQ rs6468527 A, GRM3 rs724225 G, SGMS1 rs884880 A, L3MBTL4 rs17483463 A) associated with elite endurance athlete status. These SNPs are located in the genes involved in the regulation of carbohydrate metabolism (CAMK1D), synthesis of thyroxine (CPQ), glutamatergic neurotransmission (GRM3), sphingomyelin and diacylglycerol metabolism (SGMS1) and chromatin modification (L3MBTL4).

J18.25

FREQUENCY OF ALL FORMS CONGENITAL MALFORMATIONS OF NEWBORNS BY BASED ON GENETIC REGISTER „UMIT» AND EUROPEAN INTERNATIONAL REGISTER EUROCAT

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According to the International Centre for EUROCAT, which unites more than 20 countries of the European Economic Community, the cumulative incidence of PPS varies widely - from 10.3 to 32.3 per 1,000 live births. This may be due to the peculiar environmental conditions surveyed regions, the difference in accounting methods congenital malformations, quality and principles of diagnosis, the difference in years of research. The study group was isolated malformations to be counted according to the list of the International Registry of congenital malformations (21 nosology), and analyzed in comparison with the data of our register. The overall frequency of these birth defects was 10.6 per 1,000 live births, which corresponds to the average values of the International Registry. The results of the research in our database, in comparison with the data EUROCAT more common Downs syndrome, multiple congenital malformations, congenital heart disease and malformations such as hydrocephalus, diaphragmatic hernia, Spina bifida. When comparing the frequency of congenital malformations in our case with those in the EUROCAT was found that the prevalence of a number of malformations of the central nervous system (anencephaly, encephalocele) and microtia, renal agenesis and bladder exstrophy 2-3 times lower. These types of birth defects, anophthalmia and microphthalmia as are found in our region is 3 times less than on the data recorded by the European register.

J18.26

Genome-wide association study of elite power athlete status

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The aim of the study was to identify SNPs associated with elite power athlete status in Russians using a GWAS approach (HumanOmni1-Quad BeadChips). The study involved 176 power (89 sprinters, 38 speed/strength athletes and 49 strength athletes; 102 elite and 74 sub-elite) athletes, group of athletes with speed/strength component (n=204; 64 wrestlers, 42 rugby players, 98 rowers/kayakers/canoeers), 223 endurance athletes and 173 controls. Initially, we performed seven analyses using GWAS data (elite power athletes vs. controls, all sprinters vs. controls, elite sprinters vs. controls, all speed/strength athletes vs. controls, elite speed/strength athletes vs. controls, all strength athletes vs. controls, elite strength athletes vs. controls) and found 68 SNPs which were associated with power athlete status (with P value from 0.001 to 1.345e-05) and replicated in all three subgroups of power athletes (regardless of their level of achievement). The comparison of allelic frequencies of these SNPs between the large cohort of power athletes (n=380; i.e. power athletes plus group of athletes with speed/strength component) and endurance athletes (as a second control group) resulted in remaining eight SNPs (PPARGC1B rs10060424 C, NRG1 rs17721043 A, ZNF423 rs11865138 C, RC3H1 rs767053 G, IP6K3 rs6942022 C, HSD17B14 rs7247312 G, CALCR rs17734766 G, COTL1 rs7458 T) associated with power athlete status. These SNPs are located in the genes involved in the regulation of muscle fiber composition and carbohydrate/lipid metabolism (PPARGC1B), growth and development (NRG1, ZNF423), mRNA deadenylation and degradation (RC3H1), metabolism of inositol hexakisphosphate (IP6K3), metabolism of steroids (HSD17B14), calcium homeostasis (CALCR) and actin cytoskeleton (COTL1).

J18.27

The mutation spectrum of the MEFV gene of the Southern Russia population

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Familial Mediterranean fever (FMF) is typical for populations living around the Mediterranean basin, it is also affects the Southern Russia population. Different clinical course may be caused by different mutations. Recent study reports the mutation spectrum of the FMF gene (MEFV) in the Southern Russia population.

Blood samples were collected from a cohort of 105 FMF patiens (52 male, 53 female; age: 4 to 88 years), inhabiting in the South region of Russia. The sequencing of the exon 10 in MEFV gene was performed by using a sequencer ABI PRISM 3500.

The six mutations were investigated in the exon 10 in MEFV gene in 67 patients: *M694V* - 58,3%, *V726A* - 19,1%, *M680I* - 14,8%, *R761H* - 6%, *A744S* - 0,9%, *K695R* - 0,9%.

Results of genotype analysis are shown in Table.

Genotype		Patients	%
Compound heterozygote	*M680I© / M694V*	11	16,42%
	M694V / V726A	10	14,93%
	M680I / V726A	3	4,48%
	M680I / R761H	1	1,49%
	M694V / K695R	1	1,49%
	M694V / R761H	1	1,49%
	V726A / R761H	1	1,49%
	Total	28	41,79% ± 9,32
Homozygote	*M694V / M694V*	15	22,39%
	V726A / V726A	2	2,99%
	R761H / R761H	2	2,99%
	M680I / M680I	1	1,49%
	Total	20	29,85% ± 10,23
Heterozygote	*M694V / N*	14	20,90%
	V726A / N	4	5,97%
	A744S / N	1	1,49%
	Total	19	28,36% ± 10,34
Total:		67	100%

This results can be discussed as a background to the search of new diagnostics and population research.

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J18.28

The genetic basis of the relationship between reproduction and longevity : a study on common variants of three genes in steroid hormone metabolism (CYP17, HSD17B1, COMT).

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Evolutionary theories of aging predict an antagonistic relationship between

fertility and lifespan in humans, but the genetic basis of this phenomenon is not clear. The variation of three genes in steroid hormone metabolism, CYP17 (rs743572), HSD17B1 (rs 605059) and COMT (rs4680), was examined to elucidate the genetic basis of the relationship between fertility and lifespan. A sample of 277 individuals (mean age, 82.9 years) was recruited in 2000. Based on mortality data collected in 2009, the sample was divided into two groups of subjects surviving to over 90 years (long-lived) or not (controls). Fertility data (number of children) were collected in the same sample. CYP17 and COMT gene variation did not influence either lifespan or fertility. The HSD17B1AA genotype was found to be significantly associated (p=0.0085) with longevity only in females (estimated odds ratio = 3.77). As the HSD17B1AA genotype was also associated with a higher number of children (5.3±2.1) than the other genotypes (p= 0.006), we may infer that HSD17B1 genotypes could exert a positive pleiotropic action on longevity and fertility. We then searched the literature for genes studied in relation to both reproduction and aging. A review of the studies showed a pleiotropic action for six out of sixteen genes and revealed that genes may exert positive, or negative, or antagonistic pleiotropic actions. These actions may be modified by environmental factors such as changing reproductive behaviors, which seem to be able to mitigate or to enhance the gene phenotypic effects.

J18.29

General Regression Model: a powerful "model free" association test for both qualitative and quantitative traits allowing to determine the underlying genetic model of transmission

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Genome-wide association studies (GWAS) are usually assessed using regression models, assuming additive mode of transmission. This choice leads to a significant loss of power in case of departure from additivity. We propose a General Regression Model (GRM) allowing detection of association without making hypothesis of the mode of transmission. Additionally, GRM permits the simultaneous estimation of the underlying genetic model. We compared the power of GRM to additive and other classical regression association tests under a large panel of genetic models.

Powers were estimated by simulation of a 2000 cases and 2000 controls sample for qualitative traits and a sample of 5000 cases for quantitative traits. Four association tests (GRM, additive, recessive or dominant tests) were applied to all replicates. The genetic underlying mode of transmission was then tested using GRM.

For both qualitative and quantitative trait, we showed that GRM powers to detect association was similar or greater to that of the additive test. In the case of recessive inheritance, gain of power up to 66% for qualitative and to 93% for quantitative traits respectively. The correct underlying model was determined in most cases.

GRM test appears powerful to detect association, as much as or even more than the classically used additive test, especially in case of recessive inheritance. This test is easily applied to GWAS and may be used to analyze or re-analyze new or existing GWAS datasets to identify new susceptibility loci involved in complex diseases.

J18.30

Applying the GenoChip genotyping array for tracing Indo-European expansion

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At present, the Indo-European (IE) languages form the most widespread language family. However, there is no consensus on the IE homeland, early migrations of IE speakers, and how they influenced the gene pool. We compared genetic composition of 7 pairs of geographically adjacent groups, one of which belongs to the Indo-European language family, and the other is non-Indo-European (Table). Altogether, 300 samples were genotyped with the GenoChip array which includes 130,000 autosomal, 3,000 mitochondrial, and 13,000 Y-chromosomal SNPs.

Table. The studied population pairs

The Indo-European population	**Non Indo-European population (comparison group)**
Russian	Mordvinians, Karelia
Ukrainians	Nogai and Crimean Tatars
Armenians	Georgians
Ossetians	Kabardians
Tajiks and Pamir peoples	Turkmen, Uzbeks
Lithuanians	Estonians
French and Spaniards	Basques

We ran the Principal Component (PC) analysis, compared genetic and lexico-statistical similarities, and looked for SNPs found in IE populations more frequently than in their non-IE counterparts.

PC plot shows that genetic relatedness of populations depends not only on their geographical proximity, but also on their linguistic affiliation. Though separating into European and Asian clusters follows geography, in the Asian cluster IE Pomiri and Tajiks populations formed separate cluster from Turkic-speakers, and in the European cluster IE populations are more genetically similar to each other than all European populations as a geographic group. It is notable, that in Western Europe IE Spanish and French individuals group together and differ from the non-IE Basque – the trend is opposite to that expected from geography alone.

This study was supported by the Genographic 2.0 and RFBR (grant 13-04-01711).

J18.31

FREGAT: an R library for Family REGional Association Tests

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Rapid progress in whole-exome and whole-genome sequencing technologies provides new opportunities for detecting rare variants that control complex traits. In this context, region-based association analysis is a more powerful tool for gene mapping than testing of individual genetic variants. Several approaches to regional association analysis have been developed recently and are increasingly used. However, there is still a lack of handy and powerful software tools for regional association analysis, especially for family data.

Here we present a new and effective tool for family-based regional association analysis. It offers several known high-performance regional association tests: famBT (burden test on family data based on collapsing genetic variants), FFBSKAT (fast family-based sequence kernel association test), FFBSKAT optimal, and famFLM (functional data analysis approach based on functional linear models on family data). For all these tests, the statistical properties and run-time performance have been extensively studied in recent years. High statistical power was shown under realistic simulation models, while the fast computation performance enables analysis of a whole exome within a reasonable time even on a single PC.

All tests allow for covariates and non-additive models, parallel or sequential calculation modes and run time estimation. They also offer extensive lists of specific analysis options with optimal default values. This makes FREGAT a simple, flexible, fast and effective tool for regional association analysis in related samples. The FREGAT package, along with its manual, is available for free download at http://mga.bionet.nsc.ru/soft/FREGAT/.

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J18.32

Rare coding variants and the risk of congenital anorectal malformations: an exome chip association study

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Introduction: Anorectal malformations (ARM) are rare congenital malformations and knowledge on their etiology is scarce. Previous genetic studies mainly focused on candidate genes, but did not yield substantial evidence. Hypothesis-generating approaches seem valuable for new knowledge.

Material and methods: We performed genotyping of 598 Caucasian ARM cases and 1,931 Caucasian population-based controls using the Illumina Human Exome BeadChip, which contains ~250K rare coding variants. Patients were derived from the AGORA biobank in the Netherlands and the German CURE-Net, while controls came from the Dutch Nijmegen Biomedical Study. Single variant and gene-based analyses were performed. Statistically significant single variant results ($p<1.13*10^{-6}$) were technically validated and re-

plicated in additional Caucasian and Han Chinese patients and controls with targeted sequencing, using the molecular inversion probe technique.
Results: In total, 241,177 markers passed all quality control steps. Variants with MAF>0.4% were tested (adjusted by genomic control) and 55 variants reached statistical significance in an allelic model. However, only three variants in the *CLCN1*, *LRBA*, and *ZNF423* genes could be validated and none were replicated. The gene-based analyses yielded 86 statistically significant genes, but did not lead to new insights as conditional analyses showed that gene-based signals were mainly driven by one variant per
gene, mostly already found in the single variant analyses.
Conclusions: We found no evidence for strong associations between ARM and rare coding variants captured by the HumanExome BeadChip. Future studies need large sample sizes to identify common and rare variants with small to moderate effects, while stratifying on phenotypically homogenous groups of ARM patients.

J18.33
Detection of mitochondrial haplogroups variability of small population living in 9th century based on analysis of ancient DNA

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Introduction: Ancient DNA (aDNA) represents all types of DNA that can be recovered from archaeological and palaeontological material or museum specimens. Information from aDNA is very useful in phylogenetics, paleoanthropology or genealogy. The isolation and analysis of aDNA is accompanied by two major problems: low quality and quantity of aDNA and the risk of contamination with modern DNA. Therefore, several strict laboratory and methodological criteria must be followed. The aim of this study is to isolate and analyze aDNA from human remains of the small Avar-Slavic population living in 9th century and to determine mitochondrial haplogroups in order to estimate the ratio of haplogroups typical for these two ethnicities.
Material and methods: The 50 samples of human teeth and bones were used for the isolation of aDNA in this experiment. The samples were excavated from Avar-Slavic burial site located near Cífer-Pác (Slovakia). Isolation of aDNA were performed in recommended conditions. Mitochondrial haplogroups were determined by sequencing of the HVRI of mtDNA followed by analysis of polymorphisms in this region.
Results: Despite the fact that the graves of mentioned burial place contained Avar artefacts and some remains showed mongoloid cranial features, majority of detected mitochondrial haplogroups belong to the common lineages of the Slavic populations and only presence of haplogroup U7 (typical for region of Near East) indicate the Avar origin.
Conclusion: Our results suggest that the assimilation between Avars and other neighbour ethnicities was too extensive in 9th century and, therefore the presence of haplogroups characteristic for Avars is very rare.

J18.34
Long-range modulation of PAG1 expression by 8q21 allergy risk variants

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The gene(s) whose expression is regulated by allergy risk variants is unknown for many loci identified through genome-wide association studies. Addressing this knowledge gap might point to new therapeutic targets for allergic disease. The aim of this study was to identify the target gene(s) and the functional variant(s) underlying the association between rs7009110 on chromosome 8q21 and allergies. Eight genes are located within 1 Mb of rs7009110. Multivariate association analysis of publicly available exon expression levels from lymphoblastoid cell lines (LCLs) identified a significant association between rs7009110 and the expression of a single gene: *PAG1* (P=0.0017), 732 kb away. Analysis of histone modifications and DNase I hypersensitive sites in LCLs identified four putative regulatory elements (PREs) in the region. Chromosome conformation capture confirmed that two PREs interacted with the *PAG1* promoter, one in allele-specific fashion. To determine if these PREs were functional, LCLs were transfected with *PAG1* promoter-driven luciferase reporter constructs. PRE3 acted as a transcriptional enhancer for *PAG1* exclusively when it carried the rs2370615:C allergy predisposing allele, a variant in complete linkage disequilibrium with rs7009110. As such, rs2370615, which overlaps RelA and Oct-2 transcription factor (TF) binding in LCLs and is predicted to disrupt the motif of five Forkhead TFs, represents the putative functional variant in this locus. Our studies suggest that the risk-associated allele of rs2370615 predisposes to allergic disease by increasing *PAG1* expression which may promote B-cell activation and have a pro-inflammatory effect. Inhibition of *PAG1* expression or function may have therapeutic potential for allergic diseases.

J18.35
Heritability of the mental phenomena: evidence from a Lithuanian twin study

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The problem of inheritance of the mental phenomena has not yet been solved until now. A classical method, investigation of twins, is often used to find relative contributions from genetics and the environment to quantitative traits. The aim of this study was to investigate a possible influence of heredity on cognitive functions (by applying the Raven test) and personality traits (according to the Eysenck's theory) in twins. In total, 139 pairs of same-sex twins were investigated at the Scientific Twin Center, Lithuanian University of Health Sciences. The zygosity of the twin pairs was confirmed through genotyping with 15 molecular DNA markers. The difference in the total number of incorrect solutions between monozygotic (MZ) and dizygotic (DZ) twin pairs younger than 15 years was not significant; however, in the group older than 15 years, this difference was found to be significant. Based on the total number of incorrect solutions, the concordance in the MZ twins was greater than in the DZ twins. The same tendency was found with cumulative EPQ scores on the extraversion scale. The correlation of the evaluations of cognitive functions was stronger between adult MZ twins than between the DZ ones, confirming 90% genetic similarity of MZ twins. The twin study proved that heredity by 48% influenced cognitive functions. Among the studied personality traits (psychoticism, extraversion, and neuroticism), only extraversion demonstrated a higher concordance between MZ than between DZ twins. The twin study proved that heredity by 51% influenced the extraversion trait.

J18.36
The contribution of GJB2 gene mutations in hearing loss in Eastern Siberia (the Sakha Republic) is the highest among all studied regions of Asia

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Mutations in the GJB2 (Cx26) gene are known as a major cause of congenital hearing loss. More than 150 pathogenic mutations were identified in the GJB2 gene, and mutation spectrum and frequency vary considerably among different ethnic groups. Until now, the spectrum and frequency of mutations in the GJB2 gene were not fully described in Yakutia (the Sakha Republic) located in Eastern Siberia. The complete resequencing of promoter and coding protein-regions of the GJB2 gene was performed for the first time in 393 patients with congenital hearing impairment and in 187 normal hearing individuals living in Yakutia. In total sample (n=580) we revealed 13 allelic variants of GJB2 gene, and 8 from these were recessive mutations. We found 21 different GJB2-genotypes among 393 patients, and 10 of them were with two recessive mutations. The contribution of GJB2 gene mutations in hearing loss in the population of Yakutia (45.55%) is the highest among all previously studied regions in Asia. Three mutations: c.-23+1G>A, c.35delG, c.109G>A are the most frequent among deaf patients in Yakutia. The most common mutation for Yakut patients was c.-23+1G>A (94.2% of all mutant chromosomes), and for Russian patients - c.35delG (73.1% of all mutant chromosomes). The prevalence of congenital hearing loss caused by two recessive mutations in GJB2 gene was estimated as 2.00±0.14 per 10000 population of the Sakha Republic (Yakutia). Study was supported by the RFBR

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J18.37
The role of trade-off-like and conditional genetic effects in connections between aging, health decline and longevity

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Investigating trade-off-like and conditional effects of genes is critically important for understanding complex genetic connections between aging, health decline and longevity, and for public health. Here we discuss biological mechanisms of the trade-off-like and conditional genetic effects on health related phenotypes, using results of our ongoing research and review of published studies. We emphasize importance of better understanding such effects for success of both aging research and disease prevention. This includes situations when increased risk of a major disease due to genetic factor can be compatible with longevity and/or slower aging. Accumulating evidence suggests that such situations are common and may compromise well-intended prevention strategies that target general population rather than an individual.This research was in part supported by the NIA/NIH grants R01AG046860, P01AG043352.

J18.38
Significance of thrombophilic markers in Sickle cell disease

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Background: SCD is highly prevalent in the Sultanate of Oman. The gene in its heterozygous state is present in 6% of the indigenous Omani population. One of the complications of SCD is a thrombophilic state. Intermittant painful crisis often activate the haemostatic system with consumption and reduction of several haemostatic proteins.
Aim: To study the haemostatic alterations in SCD patients during clinical and sub clinical thrombotic manifestations.
Methods: We investigated 71 cases of SCD patients in steady state or following an episode of venous thrombosis. Plasma levels Antithrombin III,[AT] Protein S and C, Activated protein C resistance, and genetic screening of Factor V Leiden, [FVL] MTHFR mutation(C677T) and CBS mutation(844ins68) was performed in 37 patients.
Results: We observed a significant reduction in Protein S and C in 18(48.6%)and 16(43.2%) cases respectively. Ten patients(27%) showed a reduction of both Protein S and C levels. No patient showed AT deficiency or FVL. MTHFR C677T mutation was seen in 16(43.2%) cases, whereas the CBS 844ins68bp was seen in 12(32.4%) cases in heterozygous state. CBS mutation was significantly correlated with low Protein S levels, whereas the MTHFR mutation alone was not correlated with any other thrombophilic marker studied.
Discussion: This small study demonstrates variable alterations in several haemostatic markers of thrombophilia studied. However, in almost all cases, as AT levels were normal, it is likely to reflect an underlying cause rather than effect as one would have expected a reduction in the AT levels in case of consumption.

J18.39
In search of the holy grail in biogeography: From DNA to home village in less than 30 seconds.

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The search for a method that utilizes biological information to predict humans' place of origin has occupied scientists for millennia. Over the past four decades, scientists have employed genetic data to address this question with limited success. Biogeographical algorithms using next-generation sequencing data achieved an accuracy of 700 km in Europe but were inaccurate elsewhere. Here we develop the Geographic Population Structure (GPS) algorithm and demonstrate its accuracy with three datasets using 40,000-130,000 SNPs to improve this accuracy. GPS placed 83% of worldwide-individuals in their country of origin. Applied to over 200 Sardinians villagers, GPS places a quarter of them in their villages and most of the remaining within 50km of their villages. The accuracy and power of GPS to infer the biogeography of worldwide-individuals down to their country or, in some cases, village, of origin, underscore the promise of admixture-based methods for biogeography and has ramifications for genetic ancestry testing. Applications for ancient DNA will be discussed.

J18.40
The „clan-based" mutation rate of the Y-chromosome estimated from high-throughput sequencing data.

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Within last three years the high-throughput sequencing technologies yield large datasets on Y-chromosomal variation in humans. Reliable estimation of the base-substitution rate is crucial for applying these data for dating population events. To estimate the mutation rate different approaches were used: human-chimpanzee comparisons (the evolutionary approach), analysis of the deep-rooting pedigree (the genealogical approach), adjusting the autosomal mutation rates, and using archeological dates of founding migrations (the calibration approach). We applied the independent "clan-based" approach using the historical dates of life of the tribe-clan founder and number of mutations accumulated among the clan members to count the mutation rate.
We genotyped 367 representatives of the Kazakh tribe Argyn by 45 SNP and 17-STR Y-chromosomal markers and found haplogroup G1-M285 with frequency 68%. The Argyn includes around 500,000 members claiming their ancestry from Karakhoja who is historical person lived 600 years ago. We applied the BigY technology (FamilyTreeDNA, Ltd) to sequence ~11 Mb of the Y-chromosomes of representatives of the 9 clans.
The reconstructed genetic tree of these Y-chromosomes perfectly fits the genealogical tree. This finding indicates the biological reality of the genealogy thus allowing calculate the Y-chromosomal mutation rate. The SNP substitution rate was 0.78×10^-9 per bp per year, falling within the range of published rates. The STR mutation rate was 0.0022 per locus per generation, very close to the so-called genealogical rate.
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J19.01
Brief report on setting up the first Huntington's Disease Center in Romania

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In Romania, the diagnosis, care and support of patients with Huntington Disease (HD) and their families is often problematic given the absence of integrated services and multidisciplinary teams of specialists. At the moment, there is no standard practice for HD: patients and families are most often unaware of their options and commonly so do professionals who come into contact with them. By setting up an HD center we aimed to bring together a multidisciplinary team able to provide a platform for professionals and HD families that will facilitate (1) assessing and diagnosing HD; (2) providing information, social and emotional support; (3) offering genetic counselling; (4) overseeing management of symptoms; (5) giving information about research and the opportunity to take part in various research projects in HD. The establishment of this center will facilitate the transfer of know-how in our country in terms of international guidelines for the assessment, diagnosis, counseling and care. Our presentation is aimed at showing the process of setting up an HD center in Romania. We will first introduce the context our team had to take into account when setting up this center and then present some of the strengths and challenges we encountered. We will detail our most representative outcomes so far as well as our plans for the near future.

J19.02
Ehlers Danlos Syndrome National Diagnostic Service and EDS-UK, working together.

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Since the Ehlers Danlos Syndrome (EDS) National Diagnostic Service was set up in 2009 we have valued our contact with the support group and charity, EDS-UK. The last six years have seen a number of changes for both of us and through this we have managed to form strong links enabling us to work closely on a number of projects.

Perhaps fortuitously, the EDS National Diagnostic Service for rare and atypical types of EDS, was set up at a time when EDS-UK were being asked to do more for members with the rare types of EDS. With our collaboration EDS-UK have now run their second patient day for patients and families with Classical, Vascular and Kyphoscoliotic EDS.
Our frustration at the amount of literature which refers to EDS as one condition led to our recent project to produce tailored information leaflets. These leaflets have now been reviewed by EDS-UK members. Here we present these new leaflets which will be available from EDS-UK for professionals and families to learn more about the different types of EDS.

J19.03

Antenatal haemoglobinopathy screening; Iron deficiency and the alpha thalassaemias - A case study.

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Introduction
As part of the service offered by the Cardiff Sickle Cell & Thalassaemia Centre and the All Wales Medical Genetics Service, haemoglobinopathy screening is offered to all women presenting at the Antenatal Clinic with family origins outside of the UK. A case is presented which illustrates the testing and counselling pathways that are activated when iron deficiency is identified in this context.
Iron deficiency in pregnancy in a woman originally from outside the UK
Mrs A accepted haemoglobinopathy screening at the antenatal clinic. She and her husband were originally from Oman. Screening identified the presence of iron deficiency.
Iron deficiency interferes with the ability to diagnose thalassaemias haematologically. A co-existing thalassaemia (most likely alpha) could therefore not be excluded without further testing.
Due to the complexities of testing for alpha thalassaemias, and also the high prevalence of certain genotypes in some populations, testing was next offered to the father of the pregnancy.
Testing identified the father, Mr B, as a possible thalassaemia carrier with the presence of Haemoglobin H inclusions. Further molecular testing was carried out to exclude the possibility of Haemoglobin Barts hydrops fetalis or Haemoglobin H disease in the pregnancy.
Initial testing ruled out the common forms of alpha zero thalassaemia. Mr B was subsequently identified as a carrier of homozygous alpha plus thalassaemia and Mrs A as a carrier of heterozygous alpha plus thalassaemia. It was therefore possible to counsel the couple that there was no discernible risk of a significant thalassaemia disorder in the pregnancy.

J19.04

Genetic risk communication and autosomal dominant polycystic kidney disease: we need to talk

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Introduction: Individuals affected by autosomal dominant polycystic kidney disease (ADPKD) are underrepresented in genetic counselling literature. Little is known about how individuals communicate the genetic risk of this condition to their relatives. We believe this is one of the first studies designed to explore the accounts of individuals diagnosed with ADPKD to understand: how communication to at risk relatives takes place, the factors influencing this process and if support is desired to facilitate family communication.
Materials and Methods: Data were collected via questionnaire (n =16), semi-structured interviews (n = 9) and retrospective medical records review (n = 9) then subsequently analysed using thematic analysis.
Results: Participants indicated several unmet needs, the most significant of which was a lack of information. Retrospective accounts showed that they recalled little to no information about ADPKD being shared with them by medical professionals at the time of diagnosis or at subsequent follow-up appointments. The results also suggested that despite current literature proposing the benefits of early intervention to reduce or slow kidney damage, the majority of participants were not aware of this information.
Conclusion: The study emphasises how misunderstandings and lack of information about ADPKD can influence genetic risk discussion within the family. Awareness of patient accounts may enhance the services designed to educate and support individuals with this condition as well as informing medical personnel who provide care to this population.

J19.05

The Italian National External Quality Assessment programme in molecular genetic testing: the Xth round (2014) results

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The Italian National Centre for Rare Diseases, at the IstitutoSuperiore di Sanità, provides the Italian External Quality Assessment (IEQA) in genetic testing since 2001. Currently, IEQA covers three molecular genetic tests: Cystic Fibrosis (CF), Beta Thalassemia (BT), Fragile X-Syndrome both full (FXf) and pre-screening (FXp) schemes.
Since 2009 the activity has been regulated by governmental document and participation is open both to public and commercial Italian laboratories. The goal of IEQA is to ensure a good laboratories practice.
Participants received four validated DNA samples with mock demographic and mock clinical indications. We ask laboratories to analyse samples and to report data using their routine protocols. Assessors review raw data and reports, sent by laboratories, taking into account genotyping, interpretation and reporting, according to marking criteria. A category of poor performance was defined.
In 2014 round the number of participants was 71: 59, 13, 12, and 6 laboratories participated for CF, BT, FXf and FXp respectively.
Results showed complete and correct data in 86%, 92%, 92%, 100% of CF, BT, FXf and FXpanalysis respectively. ten poor performing laboratories have been registered: 8 in CF, 1 in BT and 1 in FXf.
Critical errors occurred in both genotyping and interpretation in 6 and 2 laboratories respectively in CF scheme and only in genotyping in 1 laboratory in BT and in XF schemes respectively.
Assessors focused on the interpretation of results and we can show an improvement compared with previous round. This work will show in detail all Xth round results.

J19.06

Obstetrician or midwife: who should offer pregnant couples the choice between invasive and non-invasive prenatal testing (NIPT)?

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Introduction: In the Netherlands, pregnant couples at increased risk of trisomies 21, 13 and 18 may choose between invasive prenatal diagnosis (PND) and non-invasive prenatal testing (NIPT). Pre-test counseling, essential for informed decision-making, is currently reserved to obstetricians at academic hospitals. Presumably, in the near future all pregnant couples will be offered access to NIPT, leading to an increase in the demand for pre-test counselors. Our study aims to investigate whether midwives may perform pre-test counseling as adequately as academic obstetricians.
Methods: Ten midwives from a regional first tier practice will receive a tailor-made training aimed at learning to offer pre-test counseling for NIPT and PND. Couples at an increased risk of common aneuploidies will subsequently receive pretest counseling by a midwife and fill in an online questionnaire. The same questionnaire was administered to a group of couples counselled by an academic obstetrician. Data from both groups will be compared in order to assess whether pregnant couples counselled by midwives make equally informed decisions and whether choices, motivation and psychological distress differ from pregnant couples counselled by obstetricians.
Results: The training we develop comprises both theoretical knowledge and counselling skills. Our poster will present the development and content of this training.
Discussion: This study will reveal whether midwives have comparable skills and counseling results for NIPT counseling as compared to obstetricians. If so, the training we developed may be used for this purpose. If not, empirical evidence will support why the pre-test counseling license should remain with academic obstetricians.

J19.07

Genetic diagnostics from the perspective of next generation genetic sequencing of the human genome

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The fundamental and the only purpose of genetic counseling is genetic diagnostics. This means associating certain illnesses with certain genetic mutations. This has been the main purpose of genetic methods ever since they have been used in diagnostics, no matter how big the mutations are, such as changes in the number or structure of chromosomes, monogenic illnesses or microarray diagnostics. However in all of these event, the genetic test focuses on answering one specific question.
However with the appearance of next generation genetic sequencing (WGS-whole genome sequencing, WES-whole exome sequencing) we have gained the ability to directly associate certain genetic variations with certain often complex diseases sometimes even without earlier targeting of a specific gene. A good example is personal genetic sequencing which allows an individual to figure out if he carries the risk of developing certain illnesses. Nevertheless there are countless genetic variations from harmful ones to harmless ones. Due to this fact if WGS WES are going to become the diagnostic methods of the future it is essential that we target specific genetic variation which are the cause of dieses . Nevertheless like every other scientific method, WGS and WES and even genetic diagnostics as whole, can be manipulated and misused. This is why we would like to point out certain problems that may occur and the solutions to these problems, also we would like to help out future genetic counselors.

J19.08

Lynch syndrome support day: A review

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The West Midlands Clinical Genetics Service held a support and information morning for families affected with Lynch syndrome in May 2014. This support day was was organised in the West Midlands and attended by over a 100 participants, these included partners and family members.
The day provided an opportunity for individuals and families to share experiences with one another and with us. We review the feedback received for this day including lessons learnt for a future support day. We hope this review will also be a good starting point for any other centres who wish to organise a Lynch syndrome support day locally.

J19.09

Psychiatric genetic counselling: Addressing the needs of the UK population

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Introduction: Although not routinely offered for psychiatric disorders in the UK, there is growing awareness that psychiatric genetic counselling (PGC) may serve as an important tool in bridging the current gap between psychiatric genetic information and clinical application. In addition to conventional outcomes of traditional genetic counselling - including increasing aetiological understanding and provision of recurrence risks - PGC has positive psychotherapeutic outcomes through reducing feelings of shame, stigma and guilt associated with mental illness. This ultimately facilitates adaptation to the mental illness and reduces emotional burden, improving psychiatric outcomes. However there is a lack of awareness and misconceptions existing amongst the general public about the purpose of genetic counselling, and research has shown this can influence uptake and outcomes of genetic counselling sessions. Thus a potential gap exists in the future delivery of PGC services.
Method: Quantitative methods will be employed to explore a) current understanding about aetiology of mental illness b) perceptions of genetic risk c) perceptions of and attitudes towards genetic counselling and PGC, amongst UK individuals with psychiatric illness and their relatives. This will indicate i) whether PGC may be useful to this population; and ii) whether there is hypothetical interest in receiving PGC.
Results: This study will collect empirical data to assess and inform the future application of PGC to the UK.
Conclusion: This research will provide empirical evidence of whether the UK public want and would benefit from PGC. This will help inform policy makers on how to integrate this service into the current healthcare system.

J19.10

Are women making an informed choice regarding non-invasive prenatal testing for aneuploidy?

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Non-invasive prenatal testing (NIPT) for aneuploidy is currently being evaluated for use within the NHS. Previous research has highlighted concerns around routinisation of testing because of the ease with which the test can be conducted. We adapted a measure of informed choice, originally developed for use with women being offered Down syndrome screening, specifically for use with NIPT. The measure includes questions to assess knowledge, attitude, deliberation and test uptake, delivered in a questionnaire format. An informed choice is one in which there is good knowledge, deliberation, a positive attitude and NIPT uptake, or a negative attitude and NIPT is declined. The responses are calculated to provide a yes/no answer. The measure is being distributed to women recruited across seven antenatal clinics in England taking part in a study to evaluate NIPT in the NHS (www.rapid.nhs.uk). The sample includes women with a high (1:2-1:150) or intermediate (1:151-1:1000) DSS risk. Currently, 521 questionnaires have been completed. Our results indicate that the vast majority (89%) of women are making an informed choice around NIPT. Of the 11% who were judged to have made an uninformed choice, 54% had not deliberated, 44% had insufficient knowledge, and 17% had a negative attitude. Ethnicity was found to be a significant predictor of informed choice. The high rate of informed choice is likely to reflect the importance placed on the provision of pre-test counselling in this study. It will be vital to ensure this is maintained once NIPT is offered in routine clinical practice.

J19.11

Exploring the understanding of anticipation in Myotonic Dystrophy type 1 patients and families: A pilot study

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Myotonic Dystrophy Type 1 (DM1) is a dominantly inherited muscle condition predominantly characterised by muscle weakness and the phenomenon of anticipation, whereby there is increased severity of symptoms and earlier age of onset in successive generations. Anticipation is variable, highly unpredictable and may result in the birth of children severely affected with congenital myotonic dystrophy. This adds complexity to genetic counselling which may be further complicated as some patients with DM1 also have mild learning difficulties. The aim of this study was to explore communication between health professionals and patients with DM1, the understanding of Anticipation and how this may affect decisions about reproductive options. Using qualitative research methods, five interviews were conducted and analysed with six participants who had previous genetic counselling regarding anticipation with a genetics health professional. In addition thematic analysis was used to analyse clinical letters. This study confirmed important factors within understanding such as the importance of the clinician relationship and understanding from personal experience. This study also showed that though Anticipation as a concept appears well understood in this small group, patients did not understand the terminology. In addition other concepts such as chance are largely misunderstood in patients with learning difficulties. Therefore this study identifies important areas for further research into DM1 patient understanding of genetic concepts, particularly focusing on how this may affect reproductive decisions, on a much larger scale. In addition this study highlights that there is a significant lack of research within the genetic counselling of individuals with learning difficulties. Grant References: FfWG & YLCE

J20.01

Postmortem disclosure of genetic information to family members: active or passive?

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The emergence of next-generation DNA sequencing (NGS) techniques creates new moral dilemmas, among which the question whether and to whom genetic information should be returned. Whereas consensus now exists that at least some subsets of genetic information should be disclosed

to patients, communication of genetic information to family members because of hereditary risk is less consensual. This issue becomes especially salient after the patient has died, and permission to inform relatives cannot be asked anymore.
In this paper we first identify and explain the arguments in favor of and against disclosure of genetic information to a deceased patient's relatives. The principles of beneficence, the duty to warn and the familial nature of genetic information could provide justification for postmortem disclosure. Furthermore, disclosure may have the favorable side effect of fostering a relative's positive autonomy. Postmortem disclosure could however be in conflict with the deceased's privacy. Moreover, communication of genetic information could disregard the deceased's wishes or have harmful consequences for relatives and violate a relative's interest in not knowing. Finally, postmortem disclosure may not always be feasible. When balancing these different types of arguments we conclude that there are strong justifications for postmortem disclosure. Potential objections are manifold but none of them supplies compelling fundamental reasons against postmortem disclosure. There are however important competing values that need to be taken into account. Therefore we propose a passive postmortem disclosure policy offering relatives access to genetic information if certain conditions are met.

J20.02
The sustainability of new modes of research participant recruitment

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The need for participants in biomedical research, including clinical trials and genetics studies, pushes organizations to actively promote and engage the public and patients in research. In order to overcome the current difficulties of participant recruitment, web registries and platforms are being developed to collect personal health data, and to match potential research volunteers with researchers. As a pioneer in the field, the research advocacy organization Genetic Alliance launched, in 2013, the initiative Reg4ALL (Registries for ALL). Because website, blogs and social media channels are essential to keep the momentum of public engagement, we analysed the activity of the main communication channels of promotion of Reg4ALL. We found that whereas the communication effort was substantial for the launch in April 2013 and during the following 6 months, communication has decreased dramatically since December 2013. This raises the question of how active or sustainable this initiative is? And, what does it mean for the research data that have already been collected? More pragmatically, what return of investment, if any, individuals who have uploaded their data may expect from the initiative? The case of Reg4ALL raises general questions on the responsibility of research-oriented platform providers regarding the benefits claimed for their users and on patient and public engagement in research.
This research is supported through an Erasmus Mundus scholarship from the European Commission and in part by the Swedish Foundation for Humanities and Social Sciences.

ABSTRACTS 6th INTERNATIONAL WORKSHOP ON THE HISTORY OF HUMAN GENETICS

Medical genetics in Mexico: the Origins of Cytogenetics and the Health Care System

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In this paper, I explore the origins of medical cytogenetic knowledge and practices in the 1960s and 1970s in Mexico, focusing on the work of the group headed by Salvador Armendares, who spent two years in Oxford, England, with human genetics expert Alan C. Stevenson. Upon Armendares' return from England in 1966, the first Unit for Research in Human Genetics was created at a medical setting, the Instituto Mexicano del Seguro Social, (Mexican Institute of Social Security). Soon after its creation, another Mexican physician began to work with Armendares in the implementation of cytogenetics. Some of the research projects showed the embeddedness of these researchers in both public health policy and medical care, as they tackled the effects of malnutrition on chromosome structure, child mortality, chromosome aberrations and Down syndrome. Armendares and colleagues had trained at different foreign academic institutions at many different times, and contributed to transforming hospital medical practice into a medical research discipline. By posing malnutrition, one of the main concerns of Mexican post-revolutionary governments, as both medical and genetic problem, the unit contributed to positioning cytogenetic as a medical practice and a medical research domain. I will focus on the set of institutions, physicians, practices and ideas that began to reshape medical genetics to show the major roles played by both the clinic and post-revolution public health policies in the origins of medical genetics in Mexico within a global movement to delivering the benefits of scientific knowledge to the general population.

Marcus Pembrey recalls the catalyst for the establishment of the International Federation of Human Genetic Societies

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Marcus Pembrey, past President of the European Society of Human Genetics [ESHG] (1994-1995) and Emeritus Professor of Paediatric Genetics at University College London's Institute of Child Health was interviewed in 2013 concerning aspects of his work with the Avon Longitudinal Study of Parents and Children (ALSPAC). He was ALSPAC's Director of Genetics (1989-2005) and was instrumental in incorporating genetics into the initial design of this internationally acclaimed birth cohort. During this time he worked closely with ALSPAC's Ethics and Law Committee advising on the ethical issues involved when collecting genetic material from a population sample of mothers and children.
In describing his background before his involvement with ALSPAC, Professor Pembrey comments on how his ethical values evolved by discussion of real cases at the "famous Tuesday morning meetings" at Great Ormond Street Hospital, attended by many future eminent geneticists. He also describes working with Ségolène Aymé (ESHG President 1996-1997), when he was Chair of the ESHG's Public and Professional Policy Committee (1994-1998) when European policies were developed and harmonised.
He refers to an informal breakfast meeting of European and American geneticists in 1996 when his incensed American colleagues became aware of a guidelines "ambush"; without their knowledge the World Health Organisation had commissioned the writing of global ethical guidelines from Dorothy Wertz and Kåre Berg. Within 30 minutes of this disclosure, the establishment of the International Federation of Human Genetic Societies had been approved and subsequently this federation brought out their own international guidelines.

Pontecorvo's Legacy

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Establishing Glasgow as a major centre for Genetic research is largely a consequence of the vision and enthusiasm of one man, Guido Pontecorvo (1907-1999). Ponte, as he preferred to be known, was born and educated in Pisa, and was forced to leave Italy in 1938 which allowed him to settle in Scotland. After a period of internment on the Isle of Man during the second world war, Ponte returned to Scotland and was soon appointed a lecturer in Genetics at the University of Glasgow's Zoology Department. By 1950 Ponte had established the new Department of Genetics in the Anatomy laboratories of the Anderson College building. He became a Reader in 1952, before his appointment to the new Chair of Genetics (1955-1968).
Ponte is often described as *one of the founding fathers of modern genetics*. He was one of the leading figures of his day in the study of cell genetics. He was the founder of the genetics of *Aspergillus nidulans*, a relative of *Penicillium*, and originated genetic studies in many other fungi. He was also one of the first researchers to demonstrate the divisibility of the gene by recombination.
Under his leadership the Department of Genetics thrived, becoming one of the major research centres of its day, not only allowing James Renwick and others to produce the first human gene maps, but, as the departmental visitors book reveals, also attracting key researchers from far and wide to disseminate and discuss their ideas.

Unravelling the complexity of HLA: genesis and success of the International Histocompatibility Workshops

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The Human Leukocyte Antigen supergene was included in the first list of 64 genes assigned to each human chromosome at the first Human Gene Mapping workshop in 1973. Despite its complexity, the genetic control of histocompatibility was the focus of a wide effort. Following the discovery of HLA in 1958 (by Dausset, Payne and Van Rood), in the mid-1960s, a few International Histocompatibility Workshops (IHWs) contributed to set methods and standards in the field of immunogenetics and tissue-typing. These workshops were the result of a close collaboration between groups working in Europe and United States, as well as between different scientific communities. Well before the Human Genome Project, Geneticists and physicians laid the basis for one early example of "distributed big science" in biomedical disciplines. The results of the workshops were groundbreaking: the existence of one genetic system controlling histocompatibility was shown by means of a strictly genetic approach (mostly based on linkage disequilibrium analysis) that helped the interpretation of serological data, and a quick translation of the theoretical results was possible, so that HLA tissue typing was rapidly standardized and introduced for transplantation purposes. The paper will discuss the international dimension of the HLA endeavor, highlighting the conceptual and theoretical innovations introduced within the IHWs, as well as some institutional aspects of the international development of HLA research.

The Use of Oral History to Explore the Establishment of Genetic Counselling in the GDR during the 1970s and 1980s

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I frequently use interviews with contemporary witnesses - in addition to documents of participating institutions and historic publications - as the basis for my research project on "Establishing genetic counselling in the GDR in the area of conflict between science, politics and public". Most of interviewees had worked as human geneticists at the time; others had engaged with the subject from a different perspective, for example by dealing with medical ethics. Since the political situation in the GDR was complicated, a lot of background information does not emerge in official documents. Hence oral history offers the possibility to reconstruct processes of decision-making, unveil structures of communication and discern controversial opinions. In addition, it provides access to the conditions of everyday life and the working world. Taking into account the methods of Gabriele Rosenthal and Alexander von Plato, I am currently conducting open, narrative interviews. They provide interviewees with the possibility to present the subject quite autonomously. So, the interviewee's own prioritisation and emphasis become evident, as well as any avoidance of special topics. In my talk I would like to present my experiences with this approach and address the issue of how to analyse these interviews. Furthermore I include ethical and legal aspects regarding the archiving of interviews and their use as sources for publications.

The Oral History Initiative at the National Human Genome Research Institute (NHGRI)

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As part of the archival and scholarly initiative launched by Eric Green, National Human Genome Institute (NHGRI) Director, I have been conducting oral histories (videotaped and transcribed) of key NHGRI staff and leaders in the genomics research community for nearly three years. To date, twenty oral histories have been conducted with several dozen more planned over

the next five years. These oral histories will be made part of a substantial textual and video archive which will be made public in the next two years, with significant plans being made for a documentary focusing on DNA sequencing technology development using these oral histories. These oral histories will also be edited into educational segments on topics such as personalized medicine, the scientific career, comparative genomics, 'mapping the genome', genomics and ancestry and genomics and 'race' and ethnicity. My presentation will focus on the approach and conceptualization of the questions for these oral histories and a discussion of the origins of the program and the perhaps less obvious models from which it draws- Alan Macfarlane's oral histories conducted at Cambridge, United Kingdom, which have now become YouTube staples. I will also discuss the legal/ethical issues associated with producing oral histories using United States Government time and resources as well as the challenges of interviewing not only genomic researchers but also scientific program administrators, whom I believe have a unique view of the progress of genomic science. Of particular interest to this audience as well will be a summation of a series of insights gained from the oral histories about European and British efforts in mapping and sequencing, from the origins of the Human Genome Project to more recent times.

Glasgow Contributions to the Human Gene Mapping Project, 1959-1987

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The classic approaches to human gene mapping using genetic linkage in human pedigrees (Renwick) and nondisjunction and deletion in patients with chromosomal aberrations (MAF-S) were established in 1959-61 in Pontecorvo's Department. The genetic markers yielded several genetic linkages before Renwick left for UCL in 1968. The cytogenetics lab mapped a stature gene and others associated with the Turner phenotype to Xp, and TGF and XG were mapped in XX males with X-Y illegitimate recombination to just outside the pseudoautosomal boundary, leading to the discovery of SRY 24 years later. ACP1 to chromosome 2p was the first human gene to be mapped by deletion mapping. Others followed from the lab including the loci for ABO, AK1, HP, ADA, GALT, GOTS, XG, HPAFP, TS1, and HY. Heterozygosity at many other loci excluded them from the deleted regions enabling the construction of an exclusion map. A physical map of both the pairing and non-pairing regions of the Y was made using Southern blotting and DNA markers isolated from a Y chromosome library. The first reports of the successful localisation of single copy genes by isotopic in situ hybridisation came from the cytogenetics lab in 1980, specifically the regional localisation of the alpha- and beta-globin genes, followed by kappa light chain genes to 2p. Much of the Glasgow contribution to the human mapping project derived from a cytogenetic approach based on nondisjunction and deletions associated with chromosomal syndromes.

The Enduring Puzzle of Leber's Hereditary Optic Neuropathy

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Leber's disease, currently known as Leber's hereditary optic neuropathy (LHON), is now understood to be caused by mutations in mitochondria, intracellular organelles which produce cellular energy, and is inherited maternally according to a non-Mendelian pattern of inheritance. The illness was first described in 1871 by the German ophthalmologist Theodor Leber. Although the clinical aspects of the disease were generally well-accepted, its pattern of heredity proved to be an enduring puzzle. As the Australian medical geneticist David Wallace wrote in 1970: "A cursory examination of an affected family gives one the impression of a sex-linked inheritance; closer study suggests cytoplasmic inheritance; still more detailed analysis leads to confusion." This paper traces the development of the controversial theories surrounding the inheritance of LHON until the discovery of its causative mutation in 1988 by the American geneticist Douglas Wallace. This work would not have been possible without the sequencing, in 1981, of the first part of the human genome—that of mitochondria—by Fred Sanger's research group at the Medical Research Council Laboratory of Molecular Biology in Cambridge, U.K. Yet Wallace also initially faced resistance in publishing his research linking a hereditary eye disease to a mitochondrial mutation. Unlike the case of myotonic dystrophy, where Lionel Penrose was able to successfully explain away its unusual pattern of inheritance, the international and interdisciplinary community of LHON researchers failed for decades to successfully reconcile clinical findings with contemporary theories of human inheritance. The solution to the puzzle was an unexpected one.

A critical triangulation: the combination of archival sources and oral histories in the investigation of contemporary genetics

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In this presentation, I will address how the availability of new archival sources is shaping the research strategies of historians working on late-twentieth century genetics. The recent release of Codebreakers (1) and other collections documenting human genome mapping and sequencing initiatives is leading historical research to be less dependent on oral histories, given the accessibility of archival records. Some scholars may defend that these new records make interviewing dispensable and replaceable by (allegedly) less limited historical evidence. I will challenge this interpretation by arguing that the best way to exploit the new archives is in combination with oral histories. Building on my previous work on the history of biomolecular sequencing (2), I will show that the availability of archival evidence shapes the way in which oral histories are used rather than the decision of using them or not. When centralized archives are not available, projects tend to start with oral histories which lead to other interviews and personal collections still in the hands of scientists. The newly available archives enable historians to conduct oral histories after the records have been examined, this leading to more incisive questions and the selection of additional less well-known interviewees. By reporting early results of my ongoing research on the UK Human Genome Mapping Project, I will show the potential of this critical triangulation between archival and oral sources.

(1) http://wellcomelibrary.org/collections/digital-collections/makers-of-modern-genetics/

(2) García-Sancho M. (2012) Biology, Computing and the History of Molecular Sequencing: From Proteins to DNA, 1945-2000 (Palgrave Macmillan).

Interviews with Human and Medical Geneticists

P. Harper

Between 2003 and 2014 a series of 100 recorded interviews has been made by the author with older human and medical geneticists. Most of those interviewed have been from the UK or continental Europe, but the range of countries is world-wide and the fields of work varied across genetics. Currently 95 edited transcripts are accessible in full on the www.genmedhist.org/interviews website; others are still being processed; it is hoped to add audio clips, currently available on the website only for a small number of interviews. The interview transcripts provide a detailed, though not systematic picture of developments in human genetics as this has evolved during the past 60 years, notably early human cytogenetics and human molecular genetics. They also illustrate the growth of clinical genetics services over this period. Although many of the earliest founders of the field are no longer living, the interviews provide valuable details about them since they were often the teachers and mentors of those interviewed. At a personal level the interviews give vivid portraits of the individuals involved and of their colleagues, as well as the difficulties they often had to overcome in developing their lives and work. European Society of Human Genetics is now developing a new series of recorded interviews across Europe to cover the younger generation of workers.

The Thrill of Mapping: Bridging the Gap in Postwar Human Genetics

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Historians have largely examined cytogenetics and molecular biology in separate contributions. However, as the historical analysis of genetics has begun to shift towards a focus on the late-20th century, this distinction has become increasingly impractical. This paper examines the collaborative use of cytogenetic and molecular analysis by research groups as they sought to more accurately localize various disorders in the human genome, including Prader-Willi and Fragile X syndromes. During the 1970s and 80s, a number of approaches for mapping genes were developed, which integrated long-standing cytogenetic with new molecular approaches for examining the human chromosomes. I explore how attempts to better understand the genetic basis, and improve the diagnosis, of multiple genetic disorders brought together cytogenetic and molecular techniques, leading to the development of the hybrid field of molecular cytogenetics. New techniques and approaches offered exciting opportunities to bridge the gap between microscopically visible chromosomal abnormalities and molecular level nucleotide sequences, and in the process led to increased collaboration among cytogeneticists and molecular biologists. In addition to the potential clinical value of gene localization projects, I argue that many researchers were drawn into new professional alignments by the sense of thrill associated with human

gene mapping. Throughout the 1970s and 80s, Victor McKusick, Frank Ruddle, and others regularly pointed to the excitement and satisfaction associated with exploring unknown territories and identifying new genetic loci. I demonstrate how the promise and thrill of human gene mapping played a central role in bringing research communities and approaches together during the postwar era.

The History of Human Gene Mapping: remembering the times of PCR and discovery of the MECP2 gene mutation behind Rett syndrome at UCLA and translation of genetic competences to primary and secondary care

E. Houwink
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On April 14, 2003 the National Human Genome Research Institute (NHGRI), the Department of Energy (DOE) and their partners in the International Human Genome Sequencing Consortium announced the successful completion of the Human Genome Project. How did it all begin for me? When I started my career in genetics, I was still a medical student, who wished to go to the USA (1998-2001), row and for one worked at the Department of human genetics, Gonda building at UCLA, a beautiful building, hyper modern. Together with colleagues we able to use the most advanced techniques (nt PCR for one and software on our distinctive Apple computers) in this building. These were exciting times. From all over the world parents and colleague medical specialists sent blood samples on dry ice to look for the gene which could somehow explain the phenotype in different gradations of Rett syndrome. Rett syndrome is a rare disease usually found in girls, who deteriorate around 1 or 2 years of age, loose their speech and can't walk anymore. They are sometimes called "silent angels". This is really where I found my passion for genetics and its possible implementation in patient care and discovered genetics should have a more fundamental place in medical curriculum, primary and secondary care and educational programs. December 20th 2013 I received my PhD (Cum laude) on "Training in genetics and genomics for primary care workers and have moved on to two new projects on pharmacogenetics in primary care and dissemination genetics education across 6 different countries Europe wide. Even though my career is still in the early phase, I would love to share my passion for genetics from a perspective as a general practitioner (FMD) who started her career right before the Human Genome Project was completed and human gene mapping and its implications for the clinic for the near future.

Narrating 'Geneticization': Living your genome in shifting scientific paradigms

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The chronic inflammatory bowel diseases (CIBDs) were historically a paradigmatic case of psychosomatic diseases. Now they have become more genetically explainable. What does this mean for those affected? How is this 'geneticization' of CIBDs reflected in the life histories of patients and their families? In what way can their stories contribute to a better historical understanding of shifts within the biomedical explanations of diseases in the 21st century? In our project 'The lived genome and chronic inflammatory bowel diseases' we have conducted narrative interviews with above 50 affected persons (mainly long-term patients and their families) and analyzed their narratives in relation to the changes in the explanatory models. The goal of our contribution to the 'Sixth International Workshop on History of Human Genetics' is to explore the extent to which a more symmetrical image can be drawn between the historical narratives of healthcare professionals, medical historians and those narratives of people who witnessed the paradigm changes in biomedicine first-hand – or more literally with their own bodies.

"The London/Baltimore link has been severed": Human Linkage Mapping and the Early Computerization of Genetics

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Almost two decades before the Human Genome Project was conceived, a lively research program using computers to map human genes was well underway. However, limited access to computing technology and its attendant expertise created a politicized economy of information sharing that led to the project's demise. From 1958 to 1971, the American cardiologist Victor A. McKusick and British geneticist James H. Renwick maintained a transatlantic collaboration on human genetic linkage analysis. Renwick utilized IBM mainframe computers available through McKusick at Johns Hopkins to calculate recombination fractions from large families with Mendelian disorders using likelihood methods. This practice formed a substantial link between the wartime development of blood group genetics in the UK and the postwar American research-funding boom. While the collaboration began due to UK manufacturing protectionism that prevented access to cutting-edge computing technology at the time, McKusick's institution building efforts expanded the project by enrolling a network of informal investigators keen to exploit the new technology. However, as the sole arbiter of mainframe information processing, Renwick provoked anxiety amongst his collaborators. He insisted on intricate and sensitive methods that required other investigators to provide raw data to be compiled into large data sets for processing. Although they wanted to explore potential linkages within their cohort with greater certainty, Renwick allowed focus on mapping and the establishment of a data centre to take precedence, which led to conflicts. Through this narrative, I argue that collection and computation of data became competing priorities in the development of human genetics.

Human Gene Mapping: The Mass Media Iconography of Human Genome Project in the Most Popular Greek Newspapers

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The media serve as intermediate between science and the public, framing social reality for their readers and shaping the public consciousness about science-related events. In this context, images, such as genetic map, which are used by the media, play an important role in the public communications of science. They facilitate the understanding of an, often obscure, techno scientific discourse and experimental methodologies. While the scientists are at work or their scientific triumph, they visualize microcosm by making it visible, analyze our biological structure and generally our cause through a rhetorical and ideological use of their positive or negative images of a scientific fact. For journalists, images are part of a journalist's routine used for the purposes of popularizing, concretizing and dramatizing issues, in brief for making issues both newsworthy and interesting for the relevant audiences. The Human Genome Project is one of the most important scientific events which has been covered by the media and the public attention which has received has helped to change the relationship between science and society. The purpose of this announcement is to present a review of the results of a case study that focuses on the media images of Human Genome Project in the most popular Greek newspapers and how their use has affect science communication. Specifically, we examine a series of selected photographs, sketches and drawings that surrounded / accompanied the publications which, have contributed to the development of a specific public image for Human Genome Project.

James Renwick: The First Human Genetic Maps

Kevin O'Dell
School of Life Sciences, University of Glasgow, United Kingdom

James (Jim) Harrison Renwick was a Titular Professor of Genetics at the University from 1967-1968. He joined the Department of Genetics in 1959 as a Research Fellow and was appointed to Senior Lecturer in 1960 and a Reader in 1966. He made a fundamental contribution to modern genetics, in particular to the development of human gene mapping that paved the way for the Human Genome Project.

James Renwick was educated at Sedburgh School winning a Harkness Scholarship to the University of St Andrews in 1943 where he studied medicine. In 1953, after National Service a Medical Research Council grant in Human Genetics allowed Renwick to undertake a PhD in the Galton Laboratory of University College London, studying under Lionel Sharples Penrose and John Burdon Sanderson Haldane (PhD 1956). Then, in 1958-59 Renwick worked under Victor McKusick at the Johns Hopkins Hospital Department of Human Genetics and on his return to the UK he took up a post as Research Fellow in Guido Pontecorvo's Department of Genetics at the University of Glasgow where he worked until 1968.

For a period of nearly 20 years up to the early 1970s, Renwick pioneered the use of genetic markers to map disease genes on human chromosomes, seeing this field develop from its infancy at a time when there was virtually no information on mapping human genes to a major international scientific endeavour. His Independent obituarist notes that, "His work linking the ABO blood groups and the nail-patella syndrome was seminal and is still cited as a classic in human linkage analysis" and he was behind the first generalised computer program for calculating LODs (Logarithm of Odds) for large human pedigrees.

Reflections on Ethical and Theoretical Aspects of Oral History of Human Genetics in Germany

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"The stories and insights of the leaders and pioneers can help to explore the history of human genetics."
Oral History has established in history within the last years to gain more information about the history and evolution of fields that had been set up in the 20th century. Human genetics is one of those.

- The **German legal regulations** for archives in general do not allow using personal files before the person is at least ten years deceased. Therefore interviews with contemporary witnesses can give supplementary information, but also insights that have not been written down. Those have to be comparable in structure and should regard the personal and scientific situation, but also the relations to other scientists and special experiences. Oral history is supplementary to biographical files and has the same benefits and problems like autobiographical publications.
- Using Oral History as a tool for research, **ethical issues** like human dignity and personal rights must be considered. The interview partner has to be informed and must agree, but also authorise the transcription. One should careful handle and balance this information. Also one should be aware of juridical aspects.
- **Theoretical aspects** might refer to the "tacit dimension of science" (M. Polanyi), because the interviews can give insight to those aspects that were passed on only by acting or the spoken word and are not written down. This might be a first approach to a theory of oral history.

At all, oral history is a useful tool for contemporary aspects.

Changing the Point of View: the History of Human Genetics as an Applied Science in the Federal Republic of Germany from 1945 to 1975

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"Human Genetics is both a fundamental and an applied science", so stated F. Vogel and A. Motulsky.
In the history of human genetics there was less focus on the aspect as an applied science as clinical genetics. The first approach was on the meaning of the term, the first tasks in medical genetics, the influence of human genetics on legislation and the specialisation in the Federal Republic of Germany.

- For the first time the term 'Human Genetics' was used in the 1940s. Until then f.e. "Erblichkeitslehre" characterized this field. The matter of the handbooks was the same like in English ones in the first and in further publications.
- The field of activities was focussing in the beginning on paternity tests and genetic counselling. The problems in the beginning were lack of appropriate premises, but also the unsolved question of reward.
- The increasing knowledge in human genetics had no influence on legislation in the first decades after 1945. For the first time it was important in changing the abortion law at the beginning of the 1970s.
- The Wissenschaftsrat (German Science Council) said in 1960 that a chair for Genetics is necessary at every medical faculty. This was the stimulus for the professionalization of human genetics. Furthermore it can be stated that there was a personal continuity relating to the time before 1945.

It can be concluded, in spite of the German history before 1945, human genetics was characterised by continuity focussing on it as an applied science.

A Brief History of Uncertainty in Genomic Medicine

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From antiquity, scholars have opined about the dealing with uncertainty in medicine. The field of statistics was stimulated in the 17th and 18th centuries by the need to quantify the level of uncertainty based on empiric data. The iterative nature of studying uncertainty proved an important stimulus to collect, archive and analyze more data so as to improve the power of statistical and probability estimates. In more recent times, Renée Fox formalized the recognition of uncertainty as a persistent, yet changing, attribute of medical science, research and practice and emphasized that rising expectations of the benefits of medical research were paralleled by a lowered tolerance for uncertainty. As first studied by Lippman-Hand and Fraser, in genetic counselling a patient's perception of facts can be more important to decision-making than what the facts actually are. Barton Childs was struck by the dichotomy of the biologist/geneticist depending on uncertainty to generate hypotheses versus the physician/medical geneticist needing to tolerate and even embrace uncertainty and ambiguity. Today, geneticists and genomicists face an undeniable irony: as analysis of the genome reaches a natural limit, i.e., one nucleotide change out of 6.4 billion, our ability to interpret its meaning can be severely challenged. Investigations will reduce uncertainty in genomic medicine. However, given a host of factors, such as epigenetics, gene interactions with the microbiome and with other aspects of the environment, and chance, the answer to whether uncertainty can be eliminated persists. As we increasingly know more, we are increasingly aware of what we do not know.

The First Human Genetic Map 1936

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The rediscovery of Mendel's law of heredity in 1900 fueled breeding studies of plants and animals which demonstrated the independent segregation of genetic characters during meiosis. The co-segregation of grouped characters suggested to T.H. Morgan that this behavior paralleled the behavior of chromosomes during meiotic segregation. Rare cross-over events implied a model in which the frequency of such recombination events was correlated with the physical location of genetic elements on specific chromosomes. Human genetic studies did not progress because there were many human chromosomes in each human cell and the likelihood of detecting co-segregation of two characters was minimal. The genes for different blood groups appeared to segregate independently and offered an opportunity to assess potential linkage with characters such as eye and hair color, or diseases such as hemophilia and Friedrich's ataxia. However, research groups in the US and the UK found no evidence of human genetic linkage before 1935. Julia Bell and J.B.S. Haldane from the Galton Laboratory in London then studied the segregation of two characters known to be associated with the X chromosome: hemophilia and colorblindness. Their pedigree analysis published in 1936 demonstrated close linkage of the two loci. Haldane then expanded the work to involve several other genetic characters associated with the X chromosome. Recombination frequencies were used to construct a genetic map of the human X chromosome with five defined loci. The concepts developed in this work provided the basis for linkage studies in the decades ahead until the advent of DNA technology.

Project Documenting the Development of Medical Genetics in Czechoslovakia after 1945

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Prague, Czech Republic

The main aim of the presentation is the recent project on the history of medical genetics in Czechoslovakia (Bohemia, Moravia, Slovakia). It started developing in the early 1960s, when especially the reception of the then latest discoveries made at the time official doctrine of Lysenkoism obsolete. A number of institutions, mostly related with paediatrician care, were created which focused on genetic counselling and prenatal care (Prague, Brno), on basic research in the area of immunogenetics (Prague), and, somewhat later, also cytogenetics (Prague), which was originally covered by endocrinology. Faculties of medicine in Prague and Brno and the Czechoslovak Academy of Sciences played a key role in this process. The project should: i. to map the development of medical genetics by recording authentic testimonies, which can cover the generations reaching now their 80s and 70s, ii. to establish an independent documentary collection, which gathers materials and images which would otherwise be irretrievably lost. The collection covers also the earlier history starting with 1900 and includes currently approximately 400 items (books) incl. offprints and some rare issues. The material thus collected should serve i. as a starting point for a study of the turbulent history of medical genetics in Bohemia, Moravia, and Slovakia in the twentieth century, and ii. as an accessible source for comparison with the development of medical genetics elsewhere, especially in CEE countries. Another important part of the project is the preparation of a monograph and edition of interviews concerning the history of medical genetics in Bohemia and Moravia.

Collecting Genomics at the Wellcome Library

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Since 2011 the Wellcome Library has been developing its archival holdings relating to the field of genomics. The Library now holds a widespread collection of archives from notable figures and lesser known individuals, which together provide a comprehensive record of the development of genomics in Britain in the latter half of the twentieth century. These archives were acquired through a documentation strategy-based approach, which aims to capture

the networks and collaboration behind record creation, rather than just the outputs of key individuals taken in isolation. For instance, the John Sulston archive is complemented by the archives of his close collaborators Alan Coulson and Richard Durbin, whilst the wider context of Sulston's work can be understood by exploring the archives of Carol Churcher and Michael Ashburner. The poster will showcase the Wellcome Library's genomic archive collections and explain how the collecting strategy was implemented.

You're all history! Recording the voices of modern genetics

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The History of Modern Biomedicine Research Group studies the recent history of medical sciences principally by employing oral history methodology (http://www.histmodbiomed.org) . Research is focussed in five key thematic areas, one of which is clinical genetics. Of particular note is the Witness Seminar approach. These are meetings to which a group of people are invited to discuss particular debates, discoveries or developments to which they were witnesses. These meetings are recorded and edited, and the resultant volumes made freely available on the group's website. This lecture will describe some of the highlights of genetics meetings already held and discuss some of the problems and also the potentials of this type of oral history.
This work is funded by the Wellcome Trust.

Mapping the gene mapping workshops

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The History of Modern Biomedicine Research Group convenes oral history 'Witness Seminars' with key actors in contemporary biomedical areas, which are edited for publication (www.histmodbiomed.org). In 2014, a seminar was held on Human Gene Mapping Workshops, chaired by Professor Peter Harper, principally involving UK-based scientists. Discussions ranged from the first meeting in Yale, in 1973, when the distinctive 'hands-on' format and the committee reporting structure evolved, to the 1991 meeting in London, which, with significant advances in informatics, provided the organisers with new computing challenges. Topics included the foundation of the workshops; influence of somatic cell genetics; role of non-human gene mapping; advent of DNA-based techniques; culture of collaboration in the gene mapping community; clinical applications, and the origins of the Human Genome Project and advent of sequencing technologies. Extracts from the Witness Seminar and photographs of the contributors will be presented.
This work is supported by the Wellcome Trust, and we thank Professor Peter Harper for his assistance.

Witnesses to medical genetics

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Medical genetics is a core research area for the History of Modern Biomedicine Research Group (www.histmodbiomed.org). Witness Seminars have been held and published on a wide range of topics including genetic testing, clinical genetics, cancer genetics, molecular genetics and the Human Gene Mapping Workshops. This poster will present text and photographs from several of these meetings, to illustrate the Witness Seminar approach to recording the history of modern genetics.
This work is supported by the Wellcome Trust, and we thank Professor Peter Harper for his assistance.

Critical inquiry into rare disease research in Finland – Finnish Disease Heritage in a broader historical context

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The study of rare disease began in Finland during the early 1960s. During the subsequent decades, paediatricians and clinical geneticists developed the term of Finnish Disease Heritage(FDH), which is a group of over 30, typically recessive, rare diseases which are overrepresented in the Finnish population. Initially described in 1973 the term has become an important organizing principle around which genetic counselling and research in Finland has been organized. The purpose of this presentation is to present some insight into the process of studying and interviewing Finnish researchers who have been involved in the study of rare diseases in Finland. Focusing on the historical development of the notion of FDH I will seek to ponder some of the challenges one encounters in contextualizing the development of clinical genetics in Finland into broader theoretical and historical contexts. The work of respecting the time and effort provided by informants combined with the need to conduct critical inquiry poses a number of ethical challenges. Drawing on a paper I have written, the presentation will draw on experiences of interviewing Finnish researchers on their role in the development of FDH and its subsequent significance to Finnish clinical genetics.

Neonatal Screening: a Historical-comparative Perspective

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After the first regional initiatives to screen newborns for PKU in the United Kingdom and United States in the 1960s, several European countries set up screening programmes. Though the number of disorders screened for gradually increased, European countries differed in their choices for specific screening strategies and the number of disorders screened for: from 1 to 29 (Loeber et al., 2012). For instance, in case of Congenital Hypothyroidism, the Netherlands followed Canada and several US states in the strategy used to detect both primary and central forms of CH, while most European countries concentrated on the primary forms. The Netherlands was one of the earlier countries to introduce screening for Congenital Adrenal Hyperplasia (2000). The reasons for this variety may range from health care priorities and budgets to practical considerations, organisational constraints or, perhaps, chance. Pollitt (2006) has suggested that the differences may be related to the professional background of individuals involved in policy-making. For the US the influence of patient organisations and commercial parties has been mentioned (Paul & Brosco, 2013). For the Netherlands we did not find evidence that these latter forces have played a major role. In our (poster) presentation we will highlight our research on the expansion of the Dutch neonatal screening programme after its beginning in 1974 (Loeber & Van El 2014). In addition we present an initiative to stimulate historical-comparative research on the rationale for the different choices that have been made regarding the expansion of neonatal screening in Europe.

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