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- 1 Impact of gene polymorphisms of gonadotropins and their receptors on human reproductive success.
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- 12 Short Title
- 13 Gonadotropin SNPs and human reproductive success.
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17 Abstract

18 Gonadotropins and their receptors' genes carry several single-nucleotide polymorphisms resulting in 19 endocrine genotypes modulating reproductive parameters, diseases and lifespan leading to important 20 implications for reproductive success and potential relevance during human evolution. Here we illustrate 21 common genotypes of the gonadotropins and gonadotropin receptors' genes and their clinical implications 22 in phenotypes relevant for reproduction such as ovarian cycle length, age of menopause, testosterone 23 levels, polycystic ovary syndrome and cancer. We then discuss their possible role in human reproduction 24 and adaptation to the environment. Gonadotropins and their receptors' variants are differently distributed 25 among human populations. Some hints suggest that they may be the result of natural selection occurred in 26 ancient times, increasing the individual chance of successful mating, pregnancy, and effective post-natal 27 parental cares. The gender-related differences in regulation of the reproductive endocrine systems imply 28 that many of these genotypes may lead to sex-dependent effects, increasing the chance of mating and 29 reproductive success in one sex at the expenses of the other sex. Also, we suggest that sexual conflicts 30 within the follicle-stimulating and luteinizing hormone-choriogonadotropin receptor genes contributed to 31 maintain genotypes linked to subfertility among humans. Since the distribution of polymorphic markers 32 results in a defined geographical pattern due to human migrations rather than natural selection, these 33 polymorphisms may have had only a weak impact on reproductive success. On the contrary, such 34 genotypes could acquire relevant consequences in the modern, developed societies, in which parenthood 35 attempts often occur at later age, during a short, suboptimal reproductive window, making clinical fertility 36 treatments necessary. 37

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43	Introduction.
44	Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are glycoproteins produced by the
45	pituitary regulating development and reproductive functions in both men and women. On the contrary,
46	choriogonadotropin (hCG) is the human placental hormone managing pregnancy. Gonadotropins share a
47	common α subunit together with the thyroid-stimulating hormone (TSH), while having a unique β subunit,
48	specific for the receptor located in the gonads. The FSH receptor (FSHR) and the common LH/hCG receptor
49	(LHCGR) belong to the superfamily of the G protein-coupled receptors (GPCRs). They are characterized by
50	an extracellular domain, 7 trans-membrane domains joined by 3 intra- and extra-cellular loops, and an
51	intracellular, C-terminal domain. Upon hormone binding with the extracellular portion, the intracellular
52	domain triggers the activation of multiple signaling pathways by interacting with specific molecules, such as
53	G proteins or β -arrestins (Simoni et al., 1997; Ascoli et al., 2002; Gloaguen et al., 2011).
54	Gonadotropins and their receptor genes carry several single-nucleotide polymorphisms (SNPs), resulting in
55	several genotypes differently distributed among human populations and affecting sex-related reproductive
56	features and diseases by modulating signal transduction (Casarini et al., 2011). These genotypes are
57	evolutionarily old and have accompanied humans during their ancient migrations throughout the
58	continents. However, the impact of these SNPs on human reproductive success and evolution is unclear
59	and was recently debated (Grigorova et al., 2007; Simoni and Casarini, 2014).
60	Polymorphisms of the FSHR and FSHB genes.
61	The FSHR carries about two thousands SNPs but only a few of these are known as modulators of gonadal
62	response. One of the most common FSHR polymorphisms is rs6166 (NCBI SNPs database ID;
63	http://www.ncbi.nlm.nih.gov) consisting in the nucleotide change A to G at position 2039 from the gene
64	transcription start codon (c.2039A>G), and resulting in the amino acid change N to S at position 680 of the
65	protein chain (p.N680S). rs6166 is in strong <i>linkage disequilibrium</i> with the SNP rs6165 (c.919A>G,
66	p.T307A), at least in Caucasians and Asians, resulting in two discrete FSHR isoforms. p.N680S is close to the

- 67 C-terminal intracellular region of the receptor and modulates serum FSH levels and gonadal response in
- both women and men (Lledo, et al. 2013; Grigorova et al., 2014; Simoni and Casarini, 2014). Women

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69 carriers of the p.N680S S homozygous genotype have higher serum FSH levels during the follicular phase 70 and lower progesterone levels in the luteal phase than the carriers of different genotypes, while p.N680S N 71 homozygous males are characterized by higher testes volume than p.N680S S homozygous men. It was 72 suggested that the FSHR p.N680S S variant is functionally "resistant" to FSH stimulation; The p.N680S 73 polymorphism modulates cell signaling resulting in differential gene expression and steroidogenesis in 74 cultured human lutein granulosa cells as recently demonstrated in vitro (Casarini et al., 2014). 75 76 Interestingly, the cumulative effect of p.N680S together with other FSHR polymorphisms (e.g. rs1394205; 77 -29G>A) was proposed, leading to genotypes linked with lower fertility (Casarini and Simoni, 2014; 78 Grigorova et al., 2014). The -29G>A SNP falls within the 5'-untranslated region of the FSHR gene, 29 79 nucleotide upstream the ATG codon. The in vitro transcriptional activity of the -29G>A A variant is lower 80 than that of the -29G>A G genotype in Chinese hamster ovary (CHO) cells transfected with the FSHR 81 promoter and was found to be associated with hypertension (Nakayama et al., 2006), lower estradiol levels 82 in women (Achrekar et al., 2009) and higher serum FSH levels (Achrekar et al., 2009; Grigorova et al., 2014). 83 84 The FSH β subunit is encoded by the *FSHB* gene, which carries about twenty-four SNPs, but only the 85 rs10835638 (-211G>T), located in the promoter region of the gene (-211G>T, rs10835638), was extensively 86 studied in association with serum FSH levels and reproductive parameters in males (Grigorova et al. 2008). 87 In particular, -211G>T T homozygous Baltic, Italian and German men have lower FSH levels and testis 88 volume compared to carriers of other genotypes (Grigorova et al., 2008; Tüttelmann et al., 2012; Grigorova 89 et al., 2014). The promoter region of the FSHB gene is a putative target of a transcription regulatory 90 element and is highly conserved among placental mammals (Grigorova et al. 2008), suggesting that the T 91 nucleotide at position -211 affects the FSHB gene transcription leading to low hormone levels. Interestingly,

92 the studies performed in males and females are contradictory; -211G>T T homozygous women were shown

93 to have elevated FSH, LH, and reduced progesterone levels compared with carriers of other genotypes,

94 suggesting a gender-specific, compensatory regulation of the gonadotropin secretion (Schüring et al.,

95 2013). Further elucidations may be provided by genotype-phenotype association studies focusing on the 96 cumulative effect of FSHB together with FSHR gene SNPs, revealing how they affect the sex-related 97 modulation of hormone levels and reproductive parameters. Taken together, the combination of SNPs 98 within the FSHB and FSHR genes account for a substantial proportion of the total normal phenotypic 99 variance in male and female reproductive parameters (Tüttelmann et al., 2012; La Marca et al., 2013; 100 Grigorova et al., 2014; Simoni and Casarini, 2014). 101 102 Polymorphisms of the LHCGR gene and LHB/CGB gene cluster. 103 Several inactivating mutations of the LHCGR were associated with peculiar phenotypes such as 46,XY 104 disorder of sex development (DSD), primary amenorrhea and anovulation in women (Powell et al. 2003), 105 and undescended testes and androgen deficiency in men (Simoni et al. 2008), revealing the crucial role of 106 this receptor in human sex development and reproduction. LHCGR harbors at least 300 known 107 polymorphisms but only few of them lead to relevant effects (Casarini et al., 2011). 108 109 The LHCGR variant 18insLQ, consisting in the insertion of 6 nucleotides in frame in exon 1 and falling near 110 the N-terminus of the mature receptor, was associated with early onset of breast cancer and short disease-111 free survival. This is consistent with increased LHCGR 18insLQ sensitivity and plasma membrane expression 112 (1.9 fold lower hCG half-effective concentration and 1.4 fold higher expression levels than wild-type LHCGR, 113 respectively) (Piersma et al., 2006). Interestingly, LHCGR 18insLQ has a high frequency among Northern-114 European Caucasians which are characterized by higher prevalence of breast cancer compared to other 115 ethnic groups, leading to the speculation that the LHCGR genotype may be linked to disease risk (Casarini et 116 al., 2011). 117 118 Only few other LHCGR SNPs provided significant clinical findings so far. The SNP rs2293275 (c.942G>A,

p.S312N), which falls within exon 10 of the *LHCGR* gene, might affect the trafficking and stability of the

120 receptor resulting in impaired spermatogenesis in men (Simoni et al., 2008) and increased risk of

121	developing polycystic ovary syndrome (PCOS) in women (Thathapudi et al., 2015). Lastly, the polymorphic
122	LHCGR variant rs4073366 (c.3442-20797C>G) occurr about 142 base pairs downstream of LHCGR18insLQ.
123	The C allele was associated with an approximately 3-fold increased risk of developing ovarian
124	hyperstimulation syndrome (OHSS) in adult women undergoing procedures for assisted reproduction
125	(O'Brien et al., 2013).
126	
127	Few LHB gene variants are known. The so-called "V-LH" variant was discovered in Finland and consists in
128	the double amino acid exchange p.W8R and p.I15T of LHB (Pettersson et al., 1992). V-LH shows a lower
129	circulatory half time and bioactivity in vivo than the "classical" LH, possibly compensated by increased
130	transcriptional levels of the LH beta subunit due to SNPs within the promoter LHB region, which are in
131	linkage disequilibrium with p.W8R and p.I15T (Jiang et al., 1999). Curiously, V-LH may be a protective agent

132 from symptomatic PCOS in obese women, among which it is less frequent compared to healthy women and

- 133 *non*-obese PCOS patients (Tapanainen et al., 1999).
- 134

While the genes encoding the FSHβ and LHβ are present in all vertebrates, the CGβ-coding genes exist only
in primates and equids, likely as result of repeated duplications of an ancestral *LHB* gene (Henke and
Gromoll, 2008). The human genome carries eight *CGB* genes contiguous with the LHB gene on chromosome
19; subsequently frame-shift mutations and nucleotide insertions resulted in 24 additional codons for *CGB*.
The *LHB/CGB* gene cluster spans about 40 Kbase-pairs and carries several SNPs; especially, polymorphic
variants of the *CGB5* were associated with recurrent spontaneous abortions in Chinese and Caucasian
women (Rull et al., 2008; Sun and Ji, 2014).

143 Gonadotropin variants and implications in disease and menopause

144 Although further investigations are needed to elucidate the molecular mechanisms underlying the

145 modulatory effects of SNPs within FSHR and FSHB genes on reproductive parameters and diseases, their

146 pathophysiological relevance and clinical outcomes were widely described in the literature. On the

147 148

contrary, the pathophysiological implications of SNPs belonging to the *LHCGR* gene and the *LHB/CGB* gene cluster are poorly understood.

149

150 Polycystic ovarian syndrome. PCOS is a common endocrine disorder affecting 4-10% of women in 151 reproductive-age. A wide number of candidate genes were found to be potential markers of the disease 152 (Chen et al., 2011; Shi et al., 2012). PCOS women are characterized by heterogenenous sub-fertile 153 phenotypes and related clinical features. Hyperandrogenism, metabolic syndrome, insulin resistance and 154 anovulation are some of the main clinical aspects of PCOS, which may be the result of endocrine adaptation 155 to ancestral environmental conditions (Corbett and Morin-Papunen, 2013; Casarini and Brigante, 2014). 156 Several studies searched evolutionary explanations for the origin of PCOS, suggesting that the energy 157 saving resulting from less-ovulatory reproductive systems and insulin resistant phenotypes may be 158 advantageous during seasons of food shortage or high energy demand, when indeed the anovulation risk 159 increases (Vitzthum et al., 2004; Vitzthum, 2009; Corbett and Morin-Papunen, 2013). However, theories 160 supporting natural selection of PCOS phenotypes were downsized in favor of genetic drift; this issue is still 161 debated and need further investigation (Casarini and Brigante, 2014). Gonadotropins and their receptors 162 are logical candidate genes involved in the pathogenesis of the disease due to their crucial role in 163 folliculogenesis and hormone regulation. However, conflicting data exist in the literature, because of the 164 polygenic nature of the disease and the ethnic differences in the prevalence of lifestyle-related symptoms. 165 166 Alzheimer's disease. The Alzheimer's disease is a progressive, neurodegenerative disorder characterized by

neuronal and synaptic loss, neurofibrillary tangles located in neuronal cytoplasm and deposition of amyloid
 in neuritic plaques. Genoma wide association studies (GWAS) suggested that SNPs within the *FSHR* and
 LHCGR genes may contribute to the pathogenesis of the disease (Sun et al., 2014). Especially, the
 polymorphism rs4073366 (c.161+28G>C) located within the first intron of the *LHGCR* gene was associated

171 with a protective effect from the disease risk in the male (Haasl et al., 2008).

Cancer. Gonadotropins activate multiple intra-cellular signaling pathways which may result in proliferative
or anti-apoptotic events in primary cells and cell lines; also, gonadotropin receptors are expressed in
several tumor cells (Mertens-Walker et al., 2012), thus, the possible link between hormone level and cancer
risk was proposed.

177

FSHR p.N680S was indicated as possible modulator of ovarian cancer (Yang et al., 2006; Ludwig et al., 2009)
as well as LHCGR polymorphism 18insLQ, which may be linked with breast cancer risk (Powell et al., 2003).
Some studies suggested that *LHB* SNPs are risk factors for cryptorchidism (Kaleva et al., 2005) and testicular
cancer (Elkins et al., 2003). Interestingly, SNPs within gonadotropin genes were linked to papillary thyroid
cancer risk (Schonfeld, et al. 2012), revealing possible cross-activity among these molecules and their

183 receptors.

184

185 Menopausal age. A link between menopausal age and SNPs in gonadotropins and their receptors' genes 186 was suggested, providing a wide spectrum of candidate markers and conflicting, ethnicity-related results. 187 Several loci associated with age at natural menopause were identified by meta-analyzing 22 GWAS in 188 women of European ancestry (Stolk et al., 2012, Perry et al., 2014). This statistically powerful analysis 189 identified top SNPs located within 3 out of 17 genomic regions in strong linkage disequilibrium with FSHB, 190 STARD1 and BCAR4 genes in Caucasians, suggesting that they are involved in hormonal regulation of follicle 191 recruitment and exhaustion, but further confirmation in other ethnic groups are required. Interestingly, 192 women with PCOS have a later onset of menopause compared to normo-ovulatory women (Tehrani et al., 193 2010), likely resulting from the protective effect of high anti-Mullerian hormone levels for ovarian reserve, 194 extending the reproductive lifespan in spite of less ovulatory cycles.

195

Taken together, SNPs in the gonadotropins and their receptors' genes modulate fertility of both sexes andmay affect lifespan and reproductive health.

198

199 Limitations 200 Due to the polygenic regulation and the modulatory effects of lifestyle on reproductive traits (Sharma et al., 201 2013), genotype-phenotype associations need to be well-characterized in different, appropriately sized 202 sample groups and independently confirmed to avoid methodological biases. However, the medical 203 literature often provides conflicting results. Although the link between the FSHR SNP p.N680S and serum 204 FSH levels or ovarian response was repeatedly observed (Simoni and Casarini, 2014), other studies failed to 205 find the same associations (Binder et al., 2012; Mohiyiddeen et al., 2013; Trevisan et al., 2014), suggesting 206 that the endocrine features are modulated by several factors such as age or ethnicity. However, studies 207 using suboptimal sample groups characterized by subfertility or endocrine dysfunction (e.g. pre-208 menopausal women or poor responders to gonadotropin treatments) should be carefully evaluated. Proper 209 sample sizes and combined genotype analysis are required to detect significant and clinically relevant 210 associations. For example, to unmask the effects of the p.N680S polymorphism on serum FSH levels in men, 211 a combined model taking into account the FSHB promoter SNP -211G>T may be necessary (Tüttelmann et 212 al., 2012). Association studies of polygenic traits should be replicated in different sample groups rigorously 213 established, and corroborated by in vitro evidences. Finally, mathematical corrections weighting the sample 214 size from different investigations should provide the optimal verification, therefore, meta-analyzes may be 215 a safe and reliable tool to further confirm in vivo association studies.

216

217 Population genetics of of gonadotropins and gonadotropin receptors' polymorphisms

Previous studies demonstrated that the African continent holds the highest human genetic variability worldwide (Cann et al., 2002; Ramachandran et al., 2005; Li et al., 2008). Consistently with the routes of ancient human migrations, genetic variability decreases together with the distance from Africa, and oppositely to the genetic diversity, determining the current distribution of several sex-related genetic markers (Casarini and Brigante, 2014). Since natural selection contributed poorly to the distribution of human genotypes worldwide (Li et al., 2008), it is reasonable that slightly different hormonal levels and menstrual cycle duration may have only a marginal impact on the selection of sex-related genotypes,

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compared to other, more determinant phenotypic features, such as skin pigmentation or sickle cell anemia(Liu et al., 2013).

227

228 On the other hand, a full explanation of human reproductive success may not merely rely on human 229 migrations or genetic drift, and the evolutionary role of the SNPs in gonadotropin and their receptors' 230 genes was debated (Grigorova et al., 2007; Simoni and Casarini, 2014). It was estimated that about 20% of 231 Caucasians carry a "less favorable" FSHB/FSHR genotype, in terms of serum FSH levels and FSHR expression 232 and activity, which are enriched in sub-fertile subjects previously studied (Simoni and Casarini, 2014). 233 Especially, ovarian cycle length depends, at least in part, on the combination of FSHB and FSHR genotypes, 234 which affect the sensibility threshold to FSH. This results in heterogeneity in menstrual cycle length and, 235 consequently, a theoretical difference in the total number of cycles which can be calculated in about ± 30 -236 40 ovarian cycles during the reproductive lifespan depending on the FSHR genotype. FSHR p.N680S S 237 homozygous women have longer ovarian cycle than p.N680S N homozygous women (Greb et al., 2005). In 238 fact the FSHR variant carrying the amino acid serine at position 680 is more abundant in South-Central 239 Asians and Oceanians (Simoni and Casarini, 2014) who are characterized by an overall longer cycle duration 240 than women of East Asian, European or African ancestry (Vitzhum, 2009). This is consistent with the lower 241 steroidogenic potential of the FSHR p.N680S homozygous S compared to the homozygous N genotype 242 (Casarini et al., 2014). Most importantly, this suggests that some women have a lower number of 243 ovulations for months of exposure, potentially resulting in slightly lower reproductive potential, but 244 preserving the individual from unnecessary energy expenditure to maintain overall fitness (Simoni and 245 Casarini, 2014). However, since women with low cycle variability have a higher conception rate than those 246 with longer but irregular cycle duration, pregnancy success depends on cycle quality rather than length 247 (Vitzthum, 2009).

248

Prenatal maternal investments give a key contribution in maintaining progeny (Vitzhum, 2009), suggesting
that the genotype of *LHB/CGB* gene cluster is important to optimize the birth rate across human evolution.

251 Protective effect from recurrent miscarriage was associated with some SNPs located in both the CGB5 e 252 CGB8 genes, which encode the major fraction of CGB-mRNA transcripts (Rull et al., 2008) reflecting their 253 importance in physiological adaptation to pregnancy. The genomic region embedding the CGB2, CGB5 and 254 CGB8 promoter genes is featured by high heterozygosity and increased frequencies of the derived alleles in 255 non-African populations (figure 1). On the contrary, ancestral alleles of CGB2, CGB5 and CGB8 promoter 256 genes achieve the highest frequencies among individuals of African ancestry (figure 1). Moreover, high 257 heterozygosity in non-Africans suggests that balancing selection accompanied ancient human migrations 258 (Rull et al., 2008). Taken together, this is consistent with the concept that genotypic (thus phenotypic) 259 variability improves the persistence of a population in a given habitat (Forsman, 2014), providing more 260 flexible reproductive features, such as endocrine adaptation to the new environmental conditions 261 (Cornelius et al., 2013) reasonably encountered out from Africa. Interestingly, the analysis of the LHB/CGB 262 cluster sequences from several human populations revealed selective pressures among Africans compared 263 to humans in other continents (figure 2). Cross Population Extended Haplotype Homozygosity test (XP-EHH) 264 (Sabeti et al., 2007), a measure of natural selection which takes into account the SNPs frequencies within a 265 genomic region, is higher when calculated for the LHB/CGB gene cluster of individuals from Africa 266 compared to other populations. Since African populations maintained high homozygosity for the LHB gene 267 and CGB2, CGB5 and CGB8 promoter genes (figure 1), this was likely an advantageous condition in (but not 268 out from) Africa. This conflicts with the concept that Africa, where human species arose, holds the highest 269 heterozygosity and genetic variability (Cann et al., 2002; Ramachandran et al., 2005; Li et al., 2008). Also, 270 since chorionic gonadotropin is massively produced exclusively in pregnant females, the CGB gene cluster is 271 reasonably the result of selection acting only in women, providing an interesting model to study sex-related 272 aspects of the human evolution. However, the contribution of males in the selection of LHB/CGB cluster 273 genotypes should not to be excluded, at least in Africans; paternal transmission of methylated SNPs within 274 CGB5 promoter results in the loss of bi-allelic expression, leading to failure of pregnancy by impairment of 275 placental-maternal interface (Uusküla et al., 2011). In addition, a role of certain CGB transcripts in the male

- 276 reproductive system was proposed (Parrott et al., 2011) suggesting that paternal inheritance of *LHB/CGB*277 cluster genotypes was important for pregnancy in daughters.
- 278

279 An evolutionary role of pregnancy may consists in protecting from disease risk due to long-term exposure 280 to physiologic pituitary gonadotropins (Meier-Abt et al., 2015) and a link between fertility and lifespan was 281 indeed observed (Kuningas et al., 2011); it is plausible, even if speculative, that a longer lifespan could 282 provide a wider reproductive window. However the impact of life duration in human evolution remains 283 unclear, since the mean life expectancy was overall less than 40 years worldwide until the beginning of the 284 twentieth century, mainly due to causes unrelated to hormonal features (e.g. infectious diseases, famines, 285 etc) (Christensen et al., 2009), thus suggesting that the reproductive lifespan had mild beneficial effects for 286 human reproduction.

287

288 Post-natal parental care is important for progeny growth, improving reproductive success (Vitzhum, 2009). 289 Since sexual behavior and fatherhood are linked to testosterone levels in men (Gettler et al. 2013), the 290 functional significance of hormonal changes in mammalian males was debated (Saltzman and Ziegler, 291 2014). While high testosterone levels favors the male in acquiring sex partners, increased paternal care was 292 associated with low testosterone levels in humans (Pollet et al., 2013; Perini et al., 2012). Therefore, 293 genotypes linked to low fertility may have provided an evolutionary advantage, especially when the 294 adaptation to new environmental factors favored the need of cooperative behaviors among kin (Apicella et 295 al., 2012), which should be plausibly strengthened during ancient migration of relatively small human 296 groups. This may explain why the relatively recent SNP variants associated with lower fertile phenotypes, 297 such as rs1394205 (-29G>A, FSHR) and rs10835638 (-211G>T, FSHB) (Grigorova et al., 2008; Tüttelmann et 298 al., 2012), have higher frequencies among Northern European and native American populations than in 299 Africa where humans arose (Simoni and Casarini, 2014). However, the current distribution of genotypes 300 evolutionarily disadvantageous among humans may be due, at least in part, to social issues, e.g. patrilineal

301 populations, which affect the genetic diversity by sex-biased transmission of reproductive success (Heyer et302 al., 2015).

303

Reproductive conflicts.

305 Intralocus sexual conflict occurs when traits encoded by the same genetic locus result in opposite effects in 306 males and females, in terms of reproductive success (Pennell and Morrow, 2013). This was experimentally 307 demonstrated in animal models, revealing that high levels of the sex hormone testosterone result in 308 different, sex-related reproductive success in the bank vole Myodes glareolus (Mills et al., 2012). In this 309 model, high testosterone levels were oppositely associated with the reproductive success of sons and 310 daughters; thus, genetic benefits of selecting reproductively successful males with high testosterone levels 311 were lost with daughters. This may explain why genetic variants linked to sub-fertile phenotypes in females 312 did not disappeared during evolution. Since risk alleles may have been maintained in a population due to 313 their beneficial effect in one sex (Gilks et al., 2014), GWAS of sex-specific reproductive disorders could be 314 improved by including both sexes, rather than separate-sex analysis. Unfortunately, sex-related genetic 315 disorders (e.g. PCOS) are usually investigated by excluding male samples. Using human genotypic data from 316 both males and females we recently observed that sexual conflict might explain the geographic distribution 317 of PCOS risk alleles and the overall constant prevalence of the disease (Casarini and Brigante, 2014). In 318 particular, we observed that genotypes linked to hyperandrogenic phenotypes could have been 319 evolutionarily favorable for males in challenging for food resources, although disadvantageous for females 320 in which they are involved in PCOS pathogenesis. PCOS markers are SNPs located within several genomic 321 regions, including FSHR and LHCGR genes (Chen et al., 2011; Shi et al., 2012); since gonadotropin receptor 322 genes are linked to testosterone levels and testes volume in men (Grigorova et al., 2014), they may be hot 323 spots for intralocus sexual conflicts by oppositely modulating the reproductive parameters in a sex-324 dependent manner.

326 Even if speculative, the evolution of the LHB/CGB gene cluster may be a case of solved intralocus sexual 327 conflict occurred via sexual dimorphism by gene duplication (Assis and Bachtrog, 2013), resulting in the 328 independent evolution of novel functions of the derived genes. In this sense, gestation and embryo 329 development in primates are controlled by several copies of the CGB gene derived from the original LHB 330 gene (Henke and Gromoll, 2008; Nagirnaja et al., 2010), which, in turn, maintains the original physiologic 331 functions exerted in development, folliculogenesis, ovulation and spermatogenesis in all animals but the 332 primates. In primates, the number of CGB genes increase together with complexity of hemochorial 333 placentation (Cole et al., 2009), revealing that they have different, widely unknown roles in pregnancy and 334 that evolved separately. The CGB1 and CGB2 genes are highly conserved in humans and great apes, and a 335 low number of SNPs maps in the proximity of these genes. Due to the low genetic variation of CGB1 and 336 CGB2 genes, it is plausible that they are dedicated to the regulation of delicate stages such as embryo 337 implantation and placental development (Hallast et al., 2007), which are crucial for pregnancy in all 338 primates. Other CGB genes are abundantly transcribed in different gestational periods, suggesting that they 339 may serve for further, species-specific adaptations to later stages of pregnancy.

340

341 Phylogenesis

342 Due to the polygenic modulation of the sexual features, it is overall difficult to quantify the real impact of 343 each genotypic variant of the gonadotropins and their receptors' genes in human reproductive success 344 (Casarini et al., 2011). The overall, worldwide distribution of genotypic markers results in a geographical 345 pattern due to human migrations rather than selection (Ramachandran et al., 2005; Li et al., 2008). Human 346 phylogenetic trees produced using SNP frequencies of the whole FSHR and LHCGR genes from the HapMap 347 database (International HapMap Consortium, 2003) by the POPTREE2 software (Takezaki et al., 2010) 348 (figure 3) revealed indeed that the genotypic variants of both the genes are embedded in continent-specific 349 groups, depending on the genetic ancestry of the populations (Jia et al., 2014). This suggests that human 350 populations may be represented by three main FSHR and LHCGR genotypes peculiar of Africa, Eurasian and 351 East Asian-American continents, supporting that ancient human migrations gave the main contribution to

the current genetic diversity. This analysis did not take into account that few SNPs may have contributed to the selection of peculiar phenotypes (e.g. FSHR p.N680S; rs6166) more than others (e.g. non-synonymous or intronic polymorphic variants). However, the *FSHR* and *LHCGR* genes are characterized by genomic regions in high *linkage disequilibrium* (Simoni and Casarini, 2014), except in Africans, suggesting that they were inherited together. Taken together, gonadotropin receptor gene variants seem to have accompanied humans during ancient migrations only weakly contributing to their reproductive success.

358

359 Socio-economic and cultural aspects of human reproduction.

360 It is unclear how the endocrine genotypes and phenotypes affect human reproductive success in the 361 modern, developed societies, in which family structure, lifestyle and healthcare deeply changed during the 362 last century and appear now profoundly different from those of ancient times. Currently, different world 363 regions differ widely in fertility rate. The number of births per woman is inversely related with socio-364 economical indexes (per capita income, health expenditure and life expectancy) (figure 4), so that highest 365 income countries have the lowest fertility rate and this is not depending on ethnicity (data available at the 366 World Bank Group website; http://www.worldbank.org). In low income countries the mean fertility rate 367 achieves 6-8 births per woman. This means that reproductive success in current, developed human 368 societies is merely depending on social and cultural aspects reflected by richness, health, trust in the future, 369 etc., while it is poorly affected by the endocrine phenotype of the individuals. Couples of developed 370 countries currently begin to search fertility and parenthood at late reproductive age, e.g. 35-40 years, when 371 the reproductive success and birth rate are naturally low, mainly due to decreased ovarian reserve and/or 372 metabolic disturbances which amplify the effects of sub-fertile phenotypes. This explains why several 373 developed countries are currently characterized by population aging and demographic decline as compared 374 to high fertility rate observed in the poorest countries (Bongaarts, 2015). Therefore, the socio-economic 375 status is currently linked to reproductive success. In addition, in ancient human societies sexual activity 376 aiming at conception were concomitant with the beginning of the fertile age and persisted for longer times, 377 plausibly increasing the chance for parenthood as it continues to occur in the poorest countries. Endocrine

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378	and metabolic disorders, such as hyperandrogenism or insulin resistance, which result in sub-fertile female
379	phenotypes (Corbett and Morin-Papunen, 2013), might significantly affect fertility in the modern,
380	developed societies where the conception attempts per individual are reasonably fewer compared to the
381	ancient times. If so, then the genotypic features, irrelevant in the past, may be relevant to optimize fertility
382	management in the modern societies, when an increasing number of "reproductively aged" couples,
383	characterized by a reduced fertile window, undergo clinical treatments for assisted reproduction.
384	
385	Conclusions.
386	An increasing number of studies progressively elucidate how polymorphic variants of gonadotropins and
387	their receptors' genes modulate the human reproductive functions and diseases. Although traces of
388	selective pressure on genes related to endocrine functions were found, the effects of gonadotropins and
389	their receptors' SNPs should normally have relatively weak impact in human reproductive success. Peculiar
390	endocrine genotypes may be linked to phenotypes leading to opposite, sex-related reproductive success,
391	resulting in intralocus sexual conflicts and favoring the inheritance of alleles disadvantageous for one sex
392	through the ancient human history. Thus, individuals from both sexes and proper sample-sizes should be
393	required in GWAS and evolutionary studies in the field of reproduction. The endocrine phenotypes related
394	to sub-fertility may strengthen the decline of fertility in modern societies, in which parenthood attempts
395	are relegated in the last, short period of the fertile age.
396	
397	Declaration of interest.
398	The authors have no conflict of interests.
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402

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- 406
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Allele frequencies of SNPs within LHB/CGB gene cluster in human populations. Orange/blue bars indicate the proportion in percentage of the two alleles in the different human groups, which are represented by the colored lines in each column (please refer to the web browser for the populations order and name). The populations belonging the same geographical area were grouped as indicated on the right side of the panel. SNPs ID are shown above each column and grouped by gene. Pink panels above the bars indicate when mean SNP frequencies of African are significantly different versus that of all other continents (Kruskal-Wallis and Dunn's post-test; p<0.001); non-significant differences are indicated by green panels (exceptions: Africa versus America for SNPs rs753306 and rs3752210, p≥0.001). Data were obtained using the Human Genome Diversity Project (HGDP) selection browser (http://hgdp.uchicago.edu/cgi-bin/gbrowse/HGDP). 107x72mm (300 x 300 DPI)



Analysis of the natural selection pressure sustained by the LHB/CGB gene cluster. The measure of natural selection was inferred from the gene cluster sequences of several human populations using the XP-EHH index (Sabeti et al., 2007) and represented on the Y-axis (relative units). The name, ID and exon sequences (boxes and arrows) of each genes are indicated on the panel, in proximity of their genomic position on chromosome 19 (X-axis). Red lines corresponding to measures of natural selection of the LHB/CGB cluster in African achieve higher levels than that of other populations, indicating that stronger natural selection occurs in African compared to other populations. The population belonging the same geographical area were grouped and colored as indicated in the legend (top-left side of the panel); please refer to the web browser for the population name list (http://hgdp.uchicago.edu/cgi-bin/gbrowse/HGDP). The calculation of the XP-EHH index was performed by the proper online tool available at the HGDP selection browser website. 99x62mm (300 x 300 DPI)



Phylogenic analysis of the FSHR (A) and LHCGR (B) genes. SNPs frequencies were extracted from HapMap populations (http://hapmap.ncbi.nlm.nih.gov) and analyzed by the POPTREE2 software (Takezaki et al., 2010). The population belonging the same geographical area were grouped by colored ovals (Red=populations of African ancestry; Green=East Asian/American; Yellow=European Caucasian/Central Asian), resulting in phylogenetic pattern of both the FSHR and LHCGR genotypes according to the continental distribution of the human groups. The populations were assigned to each continents depending on the major genetic component of their ancestry (Jia et al., 2014); ASW were assumed as African, CHD as East Asian, GIH as Central Asian, CEU as Caucasian from Europe despite they are from USA residents. The measure of genetic distance Fst is indicated by the bars below the trees (relative frequency; please refer to the author's software and article for references about genetic distance); the numbers throughout the trees are percentage values representing an index of reliability of the analysis, which is assumed significantly reliable when ≥70-75 (relative units) (Takezaki et al., 2010). POPTREE2 software was used with these default settings: Fixation index (Fst) Uncorrected, NJ, Bootstrap 100000. 76x36mm (300 x 300 DPI)



Relationship between fertility rate and socio-economical current indexes in World countries. Fertility rate is represented as "birth per woman" (X-axis) and plotted against measures of socio-economic status, i.e. per capita income (A), health expenditure per capita (B) and life expectancy at birth (C) (logarithmic Y-axis). Fertility rate is inversely related to all these indexes, demonstrating that the countries in which people has high standard of living are featured by low number of births, and vice versa (linear or non-linear regression were used where appropriate as best-fitting model; p<0.005; calculation by GraphPad Prism, GraphPad Software Inc., La Jolla, CA, USA). The graphs were obtained using data available at the World Bank Group website (http://www.worldbank.org), an observer at the United Nations Development Group. 143x273mm (600 x 600 DPI)

1 Figure Legends

2

3 Figure 1: Allele frequencies of SNPs within LHB/CGB gene cluster in human populations.

4 Orange/blue bars indicate the proportion in percentage of the two alleles in the different

5 human groups, which are represented by the colored lines in each column (please refer to the

6 web browser for the populations order and name). The populations belonging the same

7 geographical area were grouped as indicated on the right side of the panel. SNPs ID are

8 shown above each column and grouped by gene. Pink panels above the bars indicate when

9 mean SNP frequencies of African are significantly different versus that of all other continents

10 (Kruskal-Wallis and Dunn's post-test; p < 0.001); non-significant differences are indicated by

11 green panels (exceptions: Africa versus America for SNPs rs753306 and rs3752210,

12 $p \ge 0.001$). Data were obtained using the Human Genome Diversity Project (HGDP) selection

13 browser (<u>http://hgdp.uchicago.edu/cgi-bin/gbrowse/HGDP</u>).

14

15 Figure 2: Analysis of the natural selection pressure sustained by the LHB/CGB gene cluster.

16 The measure of natural selection was inferred from the gene cluster sequences of several

17 human populations using the XP-EHH index (Sabeti et al., 2007) and represented on the Y-

18 axis (relative units). The name, ID and exon sequences (boxes and arrows) of each genes are

19 indicated on the panel, in proximity of their genomic position on chromosome 19 (X-axis).

20 Red lines corresponding to measures of natural selection of the LHB/CGB cluster in African

21 achieve higher levels than that of other populations, indicating that stronger natural selection

22 occurs in African compared to other populations. The population belonging the same

23 geographical area were grouped and colored as indicated in the legend (top-left side of the

24 panel); please refer to the web browser for the population name list

25 (http://hgdp.uchicago.edu/cgi-bin/gbrowse/HGDP). The calculation of the XP-EHH index

26 was performed by the proper online tool available at the HGDP selection browser website.

27

28 Figure 3: Phylogenic analysis of the FSHR (A) and LHCGR (B) genes. SNPs frequencies were extracted from HapMap populations (http://hapmap.ncbi.nlm.nih.gov) and analyzed by 29 the POPTREE2 software (Takezaki et al., 2010). The population belonging the same 30 geographical area were grouped by colored ovals (Red=populations of African ancestry; 31 Green=East Asian/American; Yellow=European Caucasian/Central Asian), resulting in 32 33 phylogenetic pattern of both the FSHR and LHCGR genotypes according to the continental 34 distribution of the human groups. The populations were assigned to each continents 35 depending on the major genetic component of their ancestry (Jia et al., 2014); ASW were 36 assumed as African, CHD as East Asian, GIH as Central Asian, CEU as Caucasian from Europe despite they are from USA residents. The measure of genetic distance Fst is indicated 37

38 by the bars below the trees (relative frequency; please refer to the author's software and

39 article for references about genetic distance); the numbers throughout the trees are percentage

40 values representing an index of reliability of the analysis, which is assumed significantly

41 reliable when \geq 70-75 (relative units) (Takezaki et al., 2010). POPTREE2 software was used

42 with these default settings: Fixation index (Fst) Uncorrected, NJ, Bootstrap 100000.

- 44 Figure 4: Relationship between fertility rate and socio-economical current indexes in World
- 45 countries. Fertility rate is represented as "birth per woman" (X-axis) and plotted against
- 46 measures of socio-economic status, i.e. per capita income (A), health expenditure per capita
- 47 (B) and life expectancy at birth (C) (logarithmic Y-axis). Fertility rate is inversely related to
- 48 all these indexes, demonstrating that the countries in which people has high standard of living
- 49 are featured by low number of births, and vice versa (linear or non-linear regression were
- 50 used where appropriate as best-fitting model; p<0.005; calculation by GraphPad Prism,
- 51 GraphPad Software Inc., La Jolla, CA, USA). The graphs were obtained using data available
- 52 at the World Bank Group website (http://www.worldbank.org), an observer at the United
- 53 Nations Development Group.