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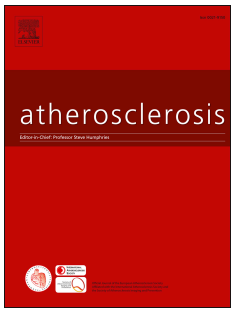

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PII: S0021-9150(15)01033-3

DOI: 10.1016/j.atherosclerosis.2015.04.815

Reference: ATH 14070

To appear in: *Atherosclerosis*

Received Date: 15 January 2015

Revised Date: 24 April 2015

Accepted Date: 26 April 2015

Please cite this article as: Rabacchi C, Pisciotta L, Cefalù AB, Noto D, Fresa R, Tarugi P, Averna M, Bertolini S, Calandra S, LPL Deficiency Study Group, Spectrum of mutations of the *LPL* gene identified in Italy in patients with severe hypertriglyceridemia, *Atherosclerosis* (2015), doi: 10.1016/j.atherosclerosis.2015.04.815.

SPECTRUM OF MUTATIONS OF THE *LPL* GENE IDENTIFIED IN ITALY IN PATIENTS WITH SEVERE HYPERTRIGLYCERIDEMIA

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Key Words: Lipoprotein lipase; primary hypertriglyceridemia; familial chylomicronemia; gene variants; pancreatitis

ABSTRACT

Background. Monogenic hypertriglyceridemia (HTG) may result from mutations in some genes which impair the intravascular lipolysis of triglyceride (TG)-rich lipoproteins mediated by the enzyme Lipoprotein lipase (LPL). Mutations in the *LPL* gene are the most frequent cause of monogenic HTG (familial chylomicronemia) with recessive transmission.

Methods. The *LPL* gene was resequenced in 149 patients with severe HTG (TG >10 mmol/L) and 106 patients with moderate HTG (TG >4.5 and <10 mmol/L) referred to tertiary Lipid Clinics in Italy.

Results. In the group of severe HTG, 26 patients (17.4%) were homozygotes, 9 patients (6%) were compound heterozygotes and 15 patients (10%) were simple heterozygotes for rare *LPL* gene variants. Single or multiple episodes of pancreatitis were recorded in 24 (48%) of these patients. There was no difference in plasma TG concentration between patients with or without a positive history of pancreatitis. Among moderate HTG patients, six patients (5.6%) were heterozygotes for rare *LPL* variants; two of them had suffered from pancreatitis. Overall 36 rare *LPL* variants were found, 15 of which not reported previously. Systematic analysis of close relatives of mutation carriers led to the identification of 44 simple heterozygotes (plasma TG 3.2 ± 4.1 mmol/L), none of whom had a positive history of pancreatitis.

Conclusions. The prevalence of rare *LPL* variants in patients with severe or moderate HTG, referred to tertiary lipid clinics, was 50/149 (33.5%) and 6/106 (5.6%), respectively. Systematic analysis of relatives of mutation carriers is an efficient way to identify heterozygotes who may develop severe HTG.

INTRODUCTION.

Lipoprotein lipase (LPL) is the enzyme, anchored to the endothelial cells of the capillaries, which is responsible for the intravascular hydrolysis of the triglycerides (TG) of TG-rich lipoproteins [1]. For full activity LPL requires co-factors such as: i) apolipoprotein C-II (apoC-II) and apolipoprotein A-V (apoA-V) which act as activators of LPL; ii) GPIHBP1 (glycosylphosphatidylinositol-anchored High Density Lipoprotein-binding Protein 1) which binds LPL in the interstitial space and transports it from the site of synthesis across the endothelial cells to the capillary lumen [1, 2]. GPIHBP1 also acts as molecular platform for LPL mediated lipolysis of TG-rich lipoproteins on the endothelial surface of the capillaries [1, 2]. In addition, the secretion of active LPL is dependent upon the activity of the Lipase Maturation Factor 1 (LMF1), an ER resident five-transmembrane protein which interacts with LPL and promotes the maturation of LPL homodimers before the exit from ER [3].

Individuals who are homozygous or compound heterozygous for loss of function mutations in the *LPL, APOC2, APOA5, GPIHBP1* or *LMF1* genes have a markedly decreased or absent LPL activity and as a consequence an impaired/delayed clearance of TG from plasma, with the accumulation of TG-rich lipoproteins (chylomicrons and VLDL) in plasma [4, 5]. The corresponding phenotype, often appearing in neonatal period/early infancy and designated familial chylomicronemia or Type I hyperlipoproteinemia, includes failure to thrive, eruptive xanthomas, lipemia retinalis, hepatosplenomegaly, recurrent abdominal pain and recurrent episodes of acute pancreatitis [6, 7]. This disorder has an estimated prevalence in the population of 1:1.000.000. Mutations in the *LPL* gene account for more than 95% of the cases of familial chylomicronemia reported so far in literature [5, 7]. Heterozygotes for *LPL* mutations have variable plasma TG levels, ranging from normal values to very high levels (>10 mmol/L) and decreased levels of high density lipoprotein-cholesterol (HDL-C) [6, 7]. Often heterozygotes develop severe hypertriglyceridemia (HTG) in association with co-morbidities, such as uncontrolled diabetes mellitus, elevated alcohol

consumption [6] or the presence of variants in other genes affecting TG metabolism [7]. The current view is that cumulative multiple genetic variants can increase the risk of HTG, especially in individuals who are heterozygous carriers of a loss of function mutation in one of the major genes affecting LPL-mediated lipolysis of TG-rich lipoproteins [8-10].

To date almost 180 *LPL* gene variants causing LPL deficiency have been reported (Supplemental Table S.10 and Supplemental references 1-53). In this study we describe the spectrum of mutations in the *LPL* gene we identified in patients with the clinical diagnosis of familial chylomicronemia or Type IV/V hyperlipidemia, referred to three Italian Lipid Clinics over the last decade.

METHODS

Hypertriglyceridemic patients

The *LPL* gene was resequenced in: i) 149 unrelated index subjects (101 males and 48 females, 38.1 ± 19.3 years of age; age range from 1 month to 77 years) with severe HTG (highest recorded plasma TG concentration >10 mmol/L: mean 27.8, median 18.4, interquartile range 13.0-26.6 mmol/L); ii) 106 unrelated subjects (94 males and 12 females, 42.9 ± 15.9 years of age; age range from 4 to 75 years) with moderate HTG (highest recorded plasma TG concentration >4.5 and <10 mmol/L: mean 7.1, median 7.0, interquartile range 5.6-8.4 mmol/L). These patients were referred to three tertiary Lipid Clinics for "primary HTG" and the clinical diagnosis of Type I (familial chylomicronemia), Type IV or Type V hyperlipidemia. Patients were Italian, with the exception of eight patients with the clinical diagnosis of familial chylomicronemia (Type I hyperlipidemia) coming from other countries (Belgium, Spain, Serbia, Panama, Equador, Morocco, Tunisia and Pakistan). In all patients the most common secondary forms of HTG (untreated/poorly controlled diabetes mellitus, obesity, alcohol abuse, chronic renal failure, HIV, use of medications and high carbohydrate diet) were excluded. The diagnosis of acute pancreatitis was based on the presence of severe epigastric pain, elevation of serum amylase and lipase of ≥ 3 times the upper limit of normal values and in most cases by characteristic findings of acute pancreatitis on contrast-enhanced computed tomography (CECT) and less commonly magnetic resonance imaging (MRI) or trans-abdominal ultrasonography.

The cohort of control subjects included 250 normolipidemic Italian subjects of both sexes. All patients, or parents in children's cases, provided informed consent for DNA analysis. The study was approved by Ethics Committees of the participating institutions. The results of this survey have been included in the data base of the recently established Italian "Lipigen Consortium".

Biochemical analysis

Plasma lipids were measured as previously specified [11]. In some patients LPL activity in post-heparin plasma was measured as reported [12]

Analysis of the *LPL* gene

Genomic DNA was extracted from peripheral blood leukocytes by a standard procedure. The exons and the promoter region of the *LPL* gene were amplified by polymerase chain reaction (PCR) and sequenced using appropriate primers [13]. Multiplex ligation-dependent probe amplification (MLPA) (SALSA MLPA P218-B1 LPL probe mix, MRC Holland, Amsterdam, the Netherlands) was used for the detection of major rearrangements of the *LPL* gene [13]. The PCR products were separated on an ABI PRISM 3100 sequencer and the data analysed by Peak Scanner™ software v1.0. Gene variants were designated according to the Human Genome Variation Society, 2013 version (http://www.hgvs.org/mutnomen/recs-DNA.html). LPL protein sequence variants were designated according to http://www.hgvs.org/mutnomen/recs-prot.html: the numerical series of codons includes the sequence of the signal peptide (27 amino acid residues).

In silico prediction of the effect of the novel missense variants of LPL was performed using PolyPhen-2 HumDiv and Hum Var (http://genetics.bwh.harvard.edu/pph2/), SIFT Human Protein and SIFT BLink (http://sift.jcvi.org/), Mutation Taster (www.mutationtaster.org), SNPs3D (www.snp3d.org) and SNAP (www.rostlab.org/services/SNAP). On the basis of in silico prediction, we arbitrarily considered the novel missense variants as pathogenic if indicated as such by 6 or 7 algorithms, probably pathogenic if indicated as such by 4 or 5 algorithms and possibly pathogenic if indicated as such by at least 3 algorithms. In silico prediction of novel splice site variants was performed using Human Splicing Finder (www.umd.be/HSF/HSF.html), NetGene2 (www.cbs.dtu.dk/service/NetGene2/) and Automated Splice Site and Exon Definition Analysis Analyses (ASSEDA) (http://splice.uwo.ca/).

Analysis of other candidate genes for HTG

The sequence of other candidate genes involved in monogenic HTG (*APOC2*, *APOA5*, *GPIHBP1*and *LMF1*) was performed as previously reported [11] in all patients carrying novel *LPL* variants and in all index patients found to be simple heterozygous for rare *LPL* variants. Besides rare variants, only the following common SNPs known to have an effect on plasma TG are reported in Tables: 1) [c.106G>A, p.(D36N) rs1801177], [c.953A>G, p.(N318S) rs268] and [c.1421C>G, p.(S474*) rs328] of the *LPL* gene; 2) -1131C>T rs662799, [c.56C>G, p.(Ser19Trp) rs3135506] of the *APOA5* gene; 3) [c.41G>T, p.(Cys14Phe) rs11538389] of the *GPIHBP1* gene [14].

Statistical analyses

Statistical analyses were performed using SPSS (PASW Statistics 18, Release Version 18.0; SPSS, Inc., 2009, Chicago, IL, www.spss.com). Differences between groups for continuous variables were assessed by Mann-Whitney test. Triglyceride levels were logarithmically transformed before analysis. Triglyceride levels (mmol/L) were reported as median and interquartile range. Differences in the distribution of categorical variables were assessed by Fisher's exact test.

RESULTS

Clinical features of homozygotes/compound heterozygotes for rare *LPL* gene variants.

Tables 1 and 2 show the list of 35 individuals with severe HTG, found to carry rare biallelic variants of the *LPL* gene in homozygous (patients 1-26) or compound heterozygous state (patients 27-35). Among the unrelated index cases 21 were homozygotes and 9 compound heterozygotes. In the latter the presence of the two variants "in trans" (i.e. on different alleles) was confirmed by sequencing the *LPL* gene in the parents.

Tables 1 and 2 also show the age at molecular diagnosis and the key clinical features of each patient. In approximately 1/3 of the patients molecular diagnosis was performed before 1 year of age. At the time of molecular diagnosis the history of a single or multiple episodes of acute pancreatitis was recorded in 19 out of 35 patients (54%).

The age of the first episode of pancreatitis (as retrieved from the clinical records) was variable from 7 months to 50 years of age (mean ± SD: 18.6 ± 15.9 years, median 17.0 years). In one patient pancreatitis occurred during pregnancy at 24 years of age. **Table 3 s**hows the mean age and plasma lipids in homozygotes/compound heterozygotes. The mean and median plasma TG levels (recorded at the time of molecular diagnosis) was 48.7 ± 57.7 mmol/L and 28.2 mmol/L, respectively (range 10.3 -326 mmol/L, interquartile range 21.1-56.7 mmol/L). There was no difference in plasma lipids between patients with or without a history of pancreatitis; pancreatitis positive patients were older than pancreatitis negative patients (**Table 4).** In children <1 year of age plasma TG level was higher than in the older children or in the adults, but the prevalence of pancreatitis was much lower (9% vs 75%)) (Supplemental Table S.1**).**

New *LPL* gene variants found in homozygotes.

Among the **21** unrelated index cases, seven were carriers of novel variants (patients 2, 3, 4, 8, 9, 23, 26) and three (patients 1, 20, 22) were carriers of two variants [**p.(G81D)** and **p.(Y329***)], reported previously by our group [15, 16] (**Table 1** and Supplemental Tables S.4A, S.5, S.6 and S.7).

The results of the multiple in silico analyses were consistent in predicting that the two novel missense variants [**p.(I109T)** and **p.(G237D)**] and that previously reported **p.(G81D)** [15] were pathogenic (Supplemental Table S.4B). These substitutions affect highly conserved amino acid residues (Supplemental Tables S.4C and S.4D).

The novel minute insertion/deletion [c.289_299delGCCGCCinsTTTGCCAAAA] and the novel minute deletion [c.651delT] (**Table 1**) cause a frameshift, predicted to result in the formation of truncated proteins devoid of function [**p.(A97F*52)** and **p.(G218Vfs*34)**].

The two novel intronic variants [**c.250-1G>C**] and [**c.1019-2A>T**] were located in the acceptor splice sites of intron 2 and intron 6, respectively. In silico algorithms (Human Splicing Finder, NetGene2 and ASSEDA) indicated that these mutations obliterated the function of the acceptor splice site. The analysis of the abnormal transcripts generated in COS-1 cells, transfected with reporter minigenes harbouring theses mutations, confirmed these predictions (Supplemental material, splice site mutation analysis).

Other homozygous patients were identified among family members of index cases (Families 9, 14, 16, 17 and 19; patients 10, 16, 19, 21 and 24, respectively). All of them had severe HTG (**Table 1)**.

No rare variants of *APOA5, APOC2, GPIHBP1* and *LMF1* were found in homozygotes carrying novel *LPL* variants. Two patients (**Table 1**, patients 8 and 26) were heterozygous carriers of the common p.(S19W) substitution in *APOA5,* and one (**Table 1**, patient 24) was homozygous carrier of the p.(C14F) substitution in *GPIHBP1*. Post-heparin plasma LPL activity measured in some patients (**Table 1**, patients 9, 11, 13, 15 and 20) was found to be <2% of the value of control plasma.

Rare *LPL* gene variants found in compound heterozygotes.

Among the 9 compound heterozygotes (15 different mutant alleles), three were carriers of five novel variants [**c.88+1G>T**, **c.88+2T>G**, **p.(E143D)**, **p.(C302S)** and **p.(H229R)**] (patients 27, 29 and 34) and one (patient 35) was a carrier of two variants [**p.(N281Mfs*23)** and ***LPL* gene**

deletion] recently described by our group [13] **(Table 2** and Supplemental Tables S.3, S.4A, S.5, S.6 and S.7)

Among the novel missense variants the **p.(E143D)** substitution was predicted to be benign or tolerated by six out of seven algorithms. However, since the genomic change (c.429G>T) underlying this missense variant involved the last nucleotide of exon 3, we thought that this mutation might affect the function of the donor splice site of intron 3. In silico analysis (Human Splicing Finder, NetGene2 and ASSEDA) indicated a substantial decrease of the function of this donor splice site (leaky site) and possibly the activation of an alternative donor site in intron 3. Thus, it is reasonable to assume that this variant generates an abnormal mRNA.

The **p.(C302S)** substitution was predicted to be not tolerated or damaging by 5 out of 7 algorithms; however, the cysteine residue at position 302 is highly conserved in various species. The **p.(H229R)** substitution was predicted to be pathogenic by all algorithms; this histidine residue is also highly conserved (Supplemental Tables S.4B, S.4C, S.4.D).

The two novel intronic variants [**c.88+1G>T** and **c.88+2T>G**], found in the same patient, affected the first and the second nucleotide respectively of the donor splice site of intron 1. In silico analysis (Human Splicing Finder, NetGene2 and ASSEDA) revealed that both mutations obliterated the function of the donor splice site of intron 1. No rare variants of *APOA5*, *APOC2*, *GPIHBP1* and *LMF1* were found in compound heterozygotes.

Heterozygous carriers of rare *LPL* gene variants

Twenty-one unrelated index cases (patients 36-56), mostly with the diagnosis of type V or type IV hyperlipidemia, were found to be simple heterozygous for rare *LPL* variants (**Table 5**). Their age ranged from 2 to 77 years (mean ± SD; 37.0 ± 21.4 years, median 41 years). Plasma TG level of these patients ranged from 4.9 to 42.1 mmo/L (median 19.4 mmol/L, interquartile range 9.2-25.4 mmol/L); in 15 patients (71.4%) plasma TG level was above 10 mmol/L. Seven of these patients (five with severe HTG - patients 45, 49, 50, 51 and 56 - and two with moderate HTG – patients 36

and 37) had a positive history of at least one episode of pancreatitis (33%); four of them had a history of recurrent pancreatitis (patients 37, 49, 51, and 56) and one subject suffered from acute pancreatitis during pregnancy (patient 50).

Fifteen different *LPL* variants were found, mostly reported previously (**Table 5**). Three novel missense variants [**p.(V64M)**, **p.(R.116Q)** and **p.(S196C)**] were identified (patients 39, 50 and 55). In silico analysis indicated that **p.(V64M)** and **p.(R116Q)** were probably pathogenic, while **p.(S196C)** was possibly pathogenic. Valine at position 64, arginine at position 116 and serine at position 196 are conserved in 12, 13 and 11 of the 13 species examined (Supplemental Tables S.4B, S.4C, S.4D). In addition one subject (patient 36) was found to be a carrier of the novel frameshift mutation (c.651delT), detected in one unrelated homozygote (patient 8 in **Table 1**).

Some patients were found to carry TG-raising SNPs of *LPL* [c.106G>A, p.(D36N) and c.953A>G, p.(N318S)], *APOA5* [-1131T>C and c.56C>G, p.(S19W)] and *GPIHBP1* [c.41C>T, p.(C14F)] (**Table 5**).

None of the rare *LPL* gene variants listed in Tables 1, 2 and 5 was found in a sample of 250 normolipidemic individuals of the Italian population.

Carriers of rare *LPL* gene variants identified in close relatives of index cases

Mutation screening among relatives of index cases (homozygotes/compound heterozygotes and simple heterozygotes) led to the identification of 44 simple heterozygotes, aged from 4 to 75 years (mean ± SD; 41.2 ± 18.0 years, median 39 years) **(Table 6**). The number of relatives per family ranged from 1 to 4. Plasma TG levels of these subjects ranged from 0.6 to 19.2 mmol/L (median 1.9 mmol/L, interquartile range 1.5-2.7 mmol/L); only in three subjects was plasma TG level above 10 mmol/L. At the age of molecular diagnosis none of these subjects had a positive history of pancreatitis.

Carriers of other *LPL* gene variants found among subjects with severe or moderate HTG

Among individuals with TG >10 mmol/L we found two brothers homozygous for a novel *LPL* variant [c.182C>T, **p.(A61V)**]. These patients (33 and 37 year-old, respectively) had a severe HTG (TG 19.1 and 18.1 mmol/L, respectively) associated with very low levels of HDL cholesterol (0.28 and 0.41 mmol/L, respectively) and undetectable LPL activity in post-heparin plasma. In silico analysis indicated that p.(A61V) was benign or tolerated and the underlying nucleotide substitution in exon 2 (c.182C>T) did not generate a new donor splice site in exon 2, which could alter the splicing process. It is possible that c.182C>T, **p.(A61V)** is in linkage with a pathogenic mutation located elsewhere in the *LPL* gene which remains elusive. For these reasons we did not include these two patients in the list of homozygotes (**Table 1**). The sequence of the other HTG related genes (*APOA5, APOC2, GPIHBP1* and *LMF1*) in these two patients did not reveal the presence of rare variants.

In the group of patients with severe HTG (TG >10 mmol/L) we found 7 carriers of common *LPL* SNPs: five heterozygotes and one homozygote for p.(D36N) and one heterozygote for p.(N318S). In these patients plasma TG level ranged from 10.3 to 25.5 mmol/L (mean ± SD: 15.9 ± 5.6 mmol/L) (Supplemental Table S.8).

Among subjects with moderate TG levels we found 9 heterozygotes and one homozygote for p.(D36N), and 10 heterozygotes for p.(N318S). Their TG levels ranged from 5.1 to 9.9 mmol/L (mean ± SD: 7.0 ± 1.5 mmol/L) (Supplemental Table S.8).

DISCUSSION

In this study we describe rare variants of the *LPL* gene found in a group of patients with severe or moderate primary hypertriglyceridemia (HTG), referred to three tertiary Lipid Clinics in Italy. The group included 149 patients with plasma TG concentration >10 mmol/L and 106 patients with plasma TG >4.5 and <10 mmol/L. Rare variants of the *LPL* gene were identified in 51 unrelated patients (21 homozygotes, 9 compound heterozygotes and 21 simple heterozygotes) (overall 20%). More specifically 11.8% of patients were carriers of two mutant alleles and 8.2% were carriers of one mutant allele.

The prevalence of monogenic LPL deficiency (presence of two mutant alleles) in the general population is estimated to be 1:1.000.000 and that of carriers of one mutant allele 1:500 [5]. The prevalence of subjects with *LPL* mutations found among patients with severe HTG varies considerably in different surveys. Wang et al. [17] found 6 carriers of rare *LPL* variants among 110 non diabetic adult patients with severe HTG (mean plasma TG level 32.6 ± 26.5 mmol/L); four of them were heterozygous for variants known to be the cause of LPL deficiency (Supplemental Table S.9) Wright et al [18] among 19 adult patients with TG >14 mmol/L found two carriers of rare LPL variants (Supplemental Table S.9). In a study of 107 adult German patients with plasma TG >10 mmol/L (mean plasma TG level 19.9 ± 13.1 mmol/L) Evans et al. [19] found 11 heterozygous carriers of deleterious *LPL* alleles (10.3%) (Supplemental Table S.9). A higher percentage of carriers of rare *LPL* variants was observed by Surendran et al. [20] in the Netherlands. They found that 34% of their patients with severe HTG referred to a tertiary Lipid Centre were carriers of *LPL* mutations, as the sole underlying cause of HTG (Supplemental Table S.9). Finally, in a recent study which included 29 patients with biochemical and/or clinical traits of chylomicronemia, Martin-Campos et al. [21] identified 6 homozygotes, 7 compound heterozygotes and 3 simple heterozygotes for rare *LPL* variants (55%) (Supplemental Table S.9). In the present study carriers of two rare *LPL* variants were found exclusively among the patients with plasma TG >10 mmol/L. By considering the unrelated index cases only (**Tables 1 and 2**), this translates into a frequency of

20%; by considering the carriers of at least one mutant allele (homozygotes, compound heterozygotes and simple heterozygotes) (**Tables 1, 2 and 5**), the percentage of carriers of rare *LPL* variants among our patients with plasma TG >10 mmol/L was approximately 30%, a figure close to that found by Surendran et al. [20]. The discrepancy between our results and those of Wang et al. [17], Wright et al [18] and Evans et al. [19] is probably due to a clinical selection bias, as our patients (who included both children and adults) have been referred to tertiary Lipid Clinics that usually investigate in depth the most severe cases of dyslipidemias especially in children and young adults. The higher percentage of patients carrying rare *LPL* variants (55%) reported by Martin-Campos et al. [21] may be explained by the young age of subjects with Type I hyperlipidemia they investigated (among 29 patients 9 were newborns and 11 children/adolescents).

The level of plasma TG showed a considerable variation in our homozygotes and compound heterozygotes, ranging from 10.3 to 326 mmol/L. Interestingly, in children below 1 year of age the mean plasmaTG level was twice that found in older children and in adult patients taken together (Supplemental Table S.1). The reason for this striking difference is not clearly understood. It probably depends on the fact that the stringent feeding schedule of breast or bottle fed infants (and the short time periods between meals) facilitate the progressive accumulation of chylomicrons over time, while in older children and in adults longer fasting periods between meals (like overnight fasting) might delay or mitigate the rate of chylomicron accumulation in plasma.

From the clinical stand point 19 homozygotes/compound heterozygotes (54%) had a positive history of at least one episode of acute pancreatitis. While pancreatitis was recorded only in 1 out of 11 patients <1 year of age, it was documented in 18 out of 24 older children and adults (age range 4-74 years), despite a lower mean level of plasma TG. In simple terms this would suggest that the longer the time of exposure to very high plasma TG levels, the higher is the chance to develop acute pancreatitis.

Among unrelated index cases found to be simple heterozygous for *LPL* variants (**Table 5**) five had moderate HTG and 15 had severe HTG. Among these patients 7 out of 21 (33%) had a positive

history of pancreatitis; in five of them plasma TG level was >10 mmol/L. In this group of patients, three (15%) had type 2 diabetes, a condition that may have contributed to increase plasma TG and worsen an otherwise mild HTG related to the presence of one mutant *LPL* allele [6, 7]. It also conceivable that patients with severe HTG were carries of variants of other HTG related genes with a cumulative effect on the phenotype [8-10]. Indeed, this appears to be the case as almost 50% of these patients were found to carry SNPs of *LPL* and other HTG related genes known to have a plasma TG raising effect (Table 5).

We performed *LPL* mutation screening in family members of index cases (homozygotes/compound heterozygotes and simple heterozygotes) and identified 44 simple heterozygotes (approximately 2 subjects per family). Their age ranged from 4 to 75 years (mean 41.2 ± 18.0; median 39.0); their TG level ranged from 0.6 to 19.2 mmol/L (mean 3.2 ± 4.1, median 1.9, interquartile range 1.5-2.7 mmol/L). The mean plasma TG level of these individuals was much lower than that recorded in the index cases found to be simple heterozygous for *LPL* mutations (**Tables 6, 7**), suggesting that in the latter other genetic or non-genetic factors contributed to HTG. None of the simple heterozygotes identified by the analysis of close relatives of index cases had a positive history of pancreatitis, probably because their plasma TG was largely below 10 mmol/L, the alert threshold level for the risk of pancreatitis; in fact, only 3 individuals (two of whom belonging the same family) had plasma TG >10 mmol/L (**Table 6).**

Overall, our observations indicate that among unrelated index cases carrying rare *LPL* gene variants with plasma TG >10 mmol/L the prevalence of pancreatitis (20/45, 44%) appears to be much higher than that (10-20%) reported in some large series of molecularly undefined hypertriglyceridemic subjects with severe HTG [22, 23].The prevalence of pancreatitis was even higher (24/50, 48%) if one considers all homozygotes (unrelated as well as related subjects), compound heterozygotes and simple heterozygotes with TG level >10 mmol/L.

As expected, we found a great allelic heterogeneity in carriers of rare *LPL* variants (**Figure 1**). Most mutations were single base changes in exons leading to 23 missense mutations (8 of which not reported previously). By using a battery of seven in silico algorithms we attempted to define the possible pathogenicity of the novel missense mutations. This analysis indicated that 4 mutations were pathogenic, 3 probably pathogenic, 1 possibly pathogenic and 1 non pathogenic (Supplemental Table S.4B). It should be stressed that seven out of eight variants involve amino acids that are highly conserved among species, suggesting that their substitution is likely to have an impact on the structure/function of the enzyme. One novel missense mutation [p.(E143D)], found in a compound heterozygote (patient 29 in Table 2), turned out to be tolerated/not deleterious. However, since the underlying genomic mutation (c.429G>T) changes the last nucleotide of exon 3, it is likely that this apparent missense mutation is in fact a splicing mutation as predicted by in silico analysis.
We found one novel nonsense mutation as well as one minute deletion and one deletion/insertion predicted to cause a frameshift leading to a premature termination codon. Finally we report four novel intronic mutations affecting splice sites. Two of them (c.250-1G>C; c.1019-2A>T) were found to generate *in vitro* abnormal mRNAs, predicted to encode truncated proteins. The other two novel mutations (c.88+1G>T and c.88+2T>G) affected the highly conserved dinucleotide “GT” of the donor splice site of intron 1 and were predicted in silico to abolish the function of this site.

One important aspect that emerged from our survey is the relatively large number of infants (<1 year of age) present in our series of homozygotes/compound heterozygotes. This is not surprising as in recent years the resequencing of the *LPL* gene (and the other monogenic HTG candidate genes) has been extended to neonates with severe HTG, as a rapid and efficient procedure to assess the presence of genetic defects of the lypolytic cascade, (replacing other more laborious assays, such as the measure of LPL activity in post-heparin plasma) [7,13, 15, 21, 24-27]. This diagnostic workup in neonates with milky plasma avoids delays in therapeutic interventions (dietary changes,

extracorporeal treatment or exchange transfusion) [13, 15, 24-27], directed to reduce plasma TG and to prevent the occurrence of acute pancreatitis.

Finally, on the basis of this survey we are now considering which of the patients listed in Tables 1 and 2 are suitable candidates for the *LPL* gene therapy (Alipogene-Tiparvovec) [28], recently approved by the European Medicines Agency for the treatment of LPL deficiency. In this context LPL activity and mass will be measured in post-heparin plasma of homozygotes/compound heterozygotes with missense mutations and a positive history of recurrent pancreatitis to select those patients, who in principle, might have the best benefit (and possibly fewer complications) from this new treatment [7].

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Conflicts of interest

The authors have no conflict of interest to disclose.

Funding Support

This study was supported by grants of the University of Genova (S.B.), the University of Modena and Reggio Emilia and Emilia-Romagna Region Rare Disease project (E35E09000880002) (S.C. and P.T.) and grants of the University of Palermo (M.A., A.B.C.) and the Italian Ministry of Research (M.A.)

LEGEND TO FIGURE

Figure 1. The figure shows the *LPL* gene mutations found in patients with severe or moderate hypertriglyceridemia identified in Italy. Novel mutations are reported in bold.

Table 1. Homozygotes for rare *LPL* gene variants identified in Lipid Clinics

Patients	Family	Age	Sex	Clinical data	TC	HDL-C	TG	LPL: homozygous mutation [genotype], proprotein
1	1	3mo	F	-	20.4	0.38	356.3	[c.242G>A], **p.(G81D)** [2]
2	2	36	M	RPC, HM	10.7	1.03	41.3	[c.250-1G>C], **p.[(T85Yfs*15, V84Efs*86)]** [1]
3	3	2mo	F	Cerebral dysmorphism	1.75	0.06	32.5	[c.289_299delGCCGCCinsTTTGCCAAAA], **p.(A97Ffs*52)** [1]
4	4	34	M	PC at 24	8.09	0.46	18.8	[c.326T>C], **p.(I109T)** [1]
5	5	74	F	RPC	6.98	0.93	21.1	[c.590G>T], p.(R197L)
6	6	7mo	F	-	9.60	0.48	56.7	[c.590G>T], p.(R197L)
7	7	53	F	PC during pregnancy	7.50	0.59	38.4	[c.644G>A], p.(G215E)
8	8	4	M	PC at 2y	5.97	0.52	18.4	[c.651delT], **p.(G218Vfs*34)** [1] / [c.56C>G], p.(S19W) (APOA5)
9	9	47	M	RPC, AH, LR	9.15	0.39	35.5	[c.710G>A], **p.(G237D)** [1]
10		44	M	PC at 39y	6.98	0.59	24.4	[c.710G>A], **p.(G237D)** [1]
11	10	11	M	SM	7.00	0.57	28.2	[c.808C>T], p.(R270C)
12	11	44	M	-.	6.45	0.82	18.4	[c.809G>A], p.(R270H)
13	12	11	F	-	4.91	0.57	23.2	[c.809G>A], p.(R270H)
14	13	48	F	HM	6.80	0.90	28.2	[c.829G>A], p.(D277N)
15	14	37	M	-	6.60	0.54	23.3	[c.835_836delCT], p.(L279Vfs*3)

16		29	F	RPC at 1-14y	5.20	0.62	16.7	[c.835_836delCT], p.(L279Vfs*3)
17	15	12	M	PC at 12y, HSM	7.75	0.45	67.8	[c.858T>A], p.(S286R)
18	16	1mo	F	LR	21.2	0.41	139.9	[c.984G>T], p.(M328I)
19		1mo	M	-	18.0	0.52	23.2	[c.984G>T], p.(M328I)
20	17	7mo	M	HSM, PC at 7mo	7.71	0.26	29.7	[c.987C>A], **p.(Y329*)** [2]
21		64	F	RPC at 50, 57, 59, 61y	5.17	0.31	24.9	[c.987C>A], **p.(Y329*)** [2]
22	18	36	F	PC at 6y, HSM, EX	6.20	0.36	28.9	[c.987C>A], **p.(Y329*)** [2]
23	19	26	M	PC at 20y	5.09	0.46	15.5	[c.1019-2A>T], **p.(V340Gfs*13)** [1]
24		20	F	PC at 6y	6.35	0.34	22.9	[c.1019-2A>T], **p.(V340Gfs*13)**[1]/ [c.41TT], p.(14FF) (GPIHBP1)
25	20	17	F	RPC since 14y, IDDM	5.82	0.77	42.7	[c.1019-3C>A], p.(V340Gfs*13)
26	21	44	F	PC at 17y	4.99	0.67	10.3	[c.1260G>A], **p.(W420*)** [1] / [c.56C>G], p.(S19W) (APOA5)

*Age (years at molecular diagnosis); M = Males; F = Females; PC = pancreatitis; RPC = recurrent pancreatitis; HM = hepatomegaly; SM = splenomegaly; HSM = hepato-splenomegaly; LR = lipemia retinalis; EX = eruptive xanthomas, AH = arterial hypertension; IDDM = insulin dependent diabetes mellitus; Plasma lipid values are in mmol/L; **Mutations in bold characters**: [1] novel mutations, [2] mutations previously identified and reported by our group.

Table 2. Compound heterozygotes for rare *LPL* gene variants identified in Lipid Clinics

Patients	Family	Age	Sex	Clinical data	TC	HDL-C	TG	LPL: mutations [genotype], proprotein
27	22	29	F	PC at 31y	12.2	0.36	124.3	[**c.88+1G>T**] + [**88+2T>G**] [1]
28	23	15	F	RPC at 6-11y	7.76	0.64	33.9	[c.264T>A], p.(Y88*) + [c.662T>C], p.(I221T)
29	24	35	M	HM	4.39	0.33	17.3	[c.429G>T], **p.(E143D)** [1] + [c.905G>C], **p.(C302S)** [1]
30	25	6mo	M	-	4.00	0.77	28.2	[c.590G>A], p.(R197H) + [c.755T>C], p.(I252T)
31	26	10mo	F	Failure to thrive	12.0	0.50	59.7	[c.644G>A], p.(G215E) + [c.701C>T], p.(P234L)
32	27	23	F	RPC at 2-9y	4.70	0.90	16.3	[c.644G>A], p.(G215E) + [c.829G>A], p.(D277N)
33	28	9mo	F	-	18.9	0.54	109.5	[c.644G>A], p.(G215E) + [c.984G>T], p.(M328I)
34	29	1mo	M	HM	12.4	0.51	64.2	[c.686A>G], **p.(H229R)** [1] + [c.829G>A], p.(D277N)
35	30	1mo	M	HM, LR, EX	17.3	0.46	140.7	[c.840delG], **p.(N281Mfs*23)** [2] + [LPL gene del], **p.0** [2]

[*]Age (years at molecular diagnosis); M = Males; F = Females; PC = pancreatitis; RPC = recurrent pancreatitis; HM = hepatomegaly; SM = splenomegaly; HSM = hepato-splenomegaly; LR = lipemia retinalis; EX = eruptive xanthomas, AH = arterial hypertension; IDDM = insulin dependent diabetes mellitus; Plasma lipid values are in mmol/L; **Mutations in bold characters**: [1] novel mutations, [2] mutations previously identified and reported by our group.

Table 3. Plasma lipid profile in homozygotes/compound heterozygotes.

	Mean ± SD	Median	Range	IQ range
Age (years)	22.7 ± 21.0	20.0	1mo-74y	8mo-37y
TC (mmol/L)	8.84 ± 5.12	6.98	1.75-21.2	5.20-10.7
HDL-C (mmol/L)	0.54 ± 0.21	0.52	0.06-1.03	0.39-0.64
TG (mmol/L)	48.7 ± 57.7	28.2	10.3-326.3	21.1-56.7

Table 4. Comparison between homozygotes/compound heterozygotes with and without history of pancreatitis.

	PC or RPC at the first episode	No PC or RCP	P*
M/F	8/11	8/8	NS
Age (years) mean ± SD	18.6 ± 15.9	11.8 ± 17.9	< 0.02
TC (mmol/L) mean ± SD	7.07 ± 1.99	10.9 ± 6.78	NS
HDL-C (mmol/L) mean ± SD	0.56 ± 0.22	0.52 ± 0.20	NS
TG (mmol/L) median, IQ range	24.9 (18.4-38.4)	30.4 (23.2-85.9)	NS

*Mann-Whitney test, Fisher exact test. PC = pancreatitis, RPC = recurrent pancreatitis.

Table 5. Simple heterozygotes for rare *LPL* gene variants not related to homozygotes/compound heterozygotes.

Patients	Family	Age*	Sex	Clinical data	TC	HDL-C	TG	LPL: mutations [genotype], proprotein
36	31	53	M	PC at 50y	5.87	0.82	6.89	[c.651delT], **p.(G218Vfs*34)** [1] / [c.56C>G], p.(S19W) (APOA5)
37	32	9	M	RPC	4.91	0.39	9.00	[c.440_443delACTA], p.(N147Tfs*24)
38	33	10	M	-	3.80	0.49	7.91	[c.984G>T], p.(M328I)
39	34	10	M	-	3.75	1.16	5.08	[c.190G>A], **p.(V64M)** [1] + [c.1421C>G], p.(S474*)
40	35	62	M	HM	8.63	0.82	14.5	[c.998G>A], p.(R333H)
41	36	47	M	-	8.49	0.63	19.6	[c.644G>A], p.(G215E) + [c.106G>A], p.(D36N) / [c.56C>G], p.(S19W) (APOA5).
42	37	12	M	-	4.63	0.77	26.0	[c.1279G>A], p.(A427T) + [c.1421C>G], p.(S474*)
43	38	2	F	-	5.01	0.50	24.7	[c.1174C>G], p.(L392V) / [c.456G>A], p.(V153M) (APOA5)
44	39	34	M	-	5.43	0.87	9.34	[c.590G>T], p.(R197L) / [c.41G>T], p.(C14F) (GPIHBP1)
45	40	48	M	T2DM, PC	9.30	1.06	39.0	[c.755T>C], p.(I252T)
46	41	32	M	T2DM	7.86	0.62	27.6	[c.829G>A], p.(D277N)
47	42	30	M	CHD	8.53	0.90	19.4	[c.829G>A], p.(D277N) / [c.56C>G], p.(S19W) (APOA5)
48	43	59	M	-	7.50	0.87	10.5	[c.829G>A], p.(D277N) + [c.953A>G], p.(N318S)

49	44	43	F	RPC	10.5	0.56	42.1	[c.829G>A], p.(D277N) / [–1131T>C] (APOA5)
50	45	38	F	PC during pregnancy	11.5	1.08	13.0	[c.347G>A], **p.(R116Q)** [1] / [–1131T>C] (APOA5)
51	46	47	F	RPC, HM, LR	9.49	0.62	22.5	[c.88+2T>G] / [c.56C>G], p.(S19W) (APOA5)
52	47	77	M	AH, CHD, HM, T2DM	7.03	0.72	22.2	[c.590G>T], p.(R197L) / [c.56C>G], p.(S19W) (APOA5)
53	48	47	M	HM	5.58	0.72	11.6	[c.829G>A], p.(D277N)
54	49	41	M	HM	9.12	0.72	24.3	[c.809G>A], p.(R270H) + [c.1421C>G], p.(S474*) / [–1131T>C] (APOA5)
55	50	10	M	HM	4.29	1.60	4.93	[c.586A>T], **p.(S196C)** [1] / [–1131T>C] (APOA5)
56	51	66	M	RPC, HM, PAD	11.1	0.67	35.6	[c.829G>A], p.(D277N) / [–1131T>C] (APOA5) / [c.41TT], p.(14FF) (GPIHBP1)

*Age (years at molecular diagnosis); M = Males; F = Females; PC = pancreatitis; RPC = recurrent pancreatitis; HM = hepatomegaly; LR = lipemia retinalis; AH = arterial hypertension; T2DM = type 2 diabetes mellitus; CHD = coronary heart disease; PAD = peripheral arterial disease; Plasma lipid values in mmol/L;

Mutations in bold characters: [1] novel mutations.

Table 6. Simple heterozygotes for rare *LPL* gene variants identified through systematic analysis of close relatives of index cases.

Family	Relatives	Age	Sex	Clinical data	TC	HDL-C	TG	LPL: mutation [genotype], proprotein
1	Father	29	M	HM	4.81	0.90	1.78	[c.242G>A], **p.(G81D)** [2]
	Mother	28	F	-	4.37	1.08	1.08	[c.242G>A], **p.(G81D)** [2]
2	Father	60	M	-	4.94	0.64	3.65	[c.250-1G>C], **p.[(T85Yfs*15, V84Efs*86)]** [1]
	Mother	58	F	-	3.98	0.82	1.82	[c.250-1G>C], **p.[(T85Yfs*15, V84Efs*86)]** [1]
3	Father	44	M	-	4.60	1.03	2.64	[c.289_299delGCCGCCinsTTTGCCAAAA], **p.(A97F*52)** [1]
	Mother	39	F	-	5.90	1.16	2.33	[c.289_299delGCCGCCinsTTTGCCAAAA], **p.(A97F*52)** [1]
8	Father	34	M	-	6.38	1.14	2.28	[c.651delT], **p.(G218Vfs*34)** [1]
	Mother	32	F	-	3.83	1.26	0.64	[c.651delT], **p.(G218Vfs*34)** [1]
9	Father	72	M	-	5.89	1.08	2.57	[c.710G>A], **p.(G237D)** [1]
	Mother	70	F	-	5.40	1.26	2.35	[c.710G>A], **p.(G237D)** [1]
	Daughter	22	F	-	4.96	1.50	2.17	[c.710G>A], **p.(G237D)** [1]
	Daughter	20	F	-	4.78	1.34	1.18	[c.710G>A], **p.(G237D)** [1]
14	Father	65	M	-	5.27	1.11	2.78	[c.835_836delCT], p.(L279Vfs*3)
	Mother	61	F	-	6.85	1.06	2.55	[c.835_836delCT], p.(L279Vfs*3)
15	Father	37	M	-	6.46	0.87	4.52	[c.858T>A], p.(S286R)
	Mother	34	F	-	4.91	1.16	1.80	[c.858T>A], p.(S286R)
19	Father	53	M	-	4.81	0.90	1.93	[c.1019-2A>T], **p.(V340Gfs*13)** [1]

	Mother	51	F	-	4.65	1.47	1.04	[c.1019-2A>T], **p.(V340Gfs*13)** [1]
22	Father	67	M	-	5.04	1.26	2.01	[c.88+1G>T]
	Mother	64	F	AH	4.83	1.24	2.61	[c.88+2T>G]
23	Father	44	M	-	5.27	1.03	2.89	[c.264T>A], p.(Y88*)
	Mother	44	F	-	5.12	1.19	2.09	[c.662T>C], p.(I221T)
24	Father	65	M	-	4.83	1.14	1.54	[c.429G>T], **p.(E143D)** [1]
	Mother	63	F	-	5.35	1.24	0.56	[c.905G>C], **p.(C302S)** [1]
25	Father	35	M	-	4.89	0.85	1.87	[c.590G>A], p.(R197H)
	Mother	28	F	-	5.43	1.14	1.42	[c.755T>C], p.(I252T)
26	Father	30	M	-	5.40	0.98	3.05	[c.701C>T], p.(P234L)
	Mother	25	F	-	4.60	1.24	1.87	[c.644G>A], p.(G215E)
27	Father	53	M	AH	5.94	1.00	2.94	[c.829G>A], p.(D277N)
	Mother	51	F	-	4.45	1.29	1.92	[c.644G>A], p.(G215E)
28	Father	34	M	-	6.38	1.26	1.21	[c.644G>A], p.(G215E)
	Mother	30	F	-	5.35	1.55	0.95	[c.984G>T], p.(M328I)
29	Father	42	M	-	6.10	0.95	1.94	[c.686A>G], **p.(H229R)** [1]
	Mother	31	F	-	4.32	1.16	1.50	[c.829G>A], p.(D277N)
30	Father	39	M	-	5.06	1.47	1.00	[LPL gene del], **p.0** [2]

	Mother	36	F	-	4.60	1.56	0.86	[c.840delG], **p.(N281Mfs*23)** [2]
33	Brother	14	M	-	4.60	0.67	7.91	[c.984G>T], p.(M328I)
45	Father	75	M	T2DM, AH	5.74	1.03	4.49	[c.347G>A], **p.(R116Q)** [1]
47	Son	48	M	AH, HM	7.00	0.67	19.2	[c.590G>T], p.(R197L)
49	Daughter	15	F	-	5.45	1.29	1.64	[c.809G>A], p.(R270H)
	Son	8	M	-	4.00	1.13	1.76	[c.809G>A], p.(R270H)
	Daughter	4	F	-	4.08	0.98	1.46	[c.809G>A], p.(R270H)
51	Daughter	41	F	-	6.44	0.83	12.5	[c.829G>A], p.(D277N)
	Grandchild	20	M	-	8.92	0.64	19.2	[c.829G>A], p.(D277N)

M = Males; F = Females; HM = hepatomegaly; AH = arterial hypertension; T2DM = type 2 diabetes mellitus; Plasma lipid values in mmol/L; **Mutations in bold characters**: [1] novel mutations, [2] mutations previously identified and reported by our group.

Table 7. Comparison between simple heterozygotes index cases and simple heterozygotes identified through systematic analysis of close relatives of index cases.

	Heterozygotes Index cases	Heterozygotes related to Index cases*	P†
M/F	17/4	22/22	0.03
Null alleles/missense	3/18	15/29	NS
Age (years) mean ± SD	37.0 ± 21.4	41.2 ± 18.0	NS
TC (mmol/L) mean ± SD	7.25 ± 2.45	5.27 ± 0.95	0.003
HDL-C (mmol/L) mean ± SD	0.79 ± 0.27	1.10 ± 0.23	0.0001
TG (mmol/L) median, IQ range	19.4 (9.2-25.4)	1.93 (1.47-2.74)	0.0001

*Identified through systematic analysis of close relatives of index cases listed in Tables 1, 2 and 5.

†Fisher exact test and Mann-Whitney test.

LPL gene (8p22) mutations found in Lipid Clinics

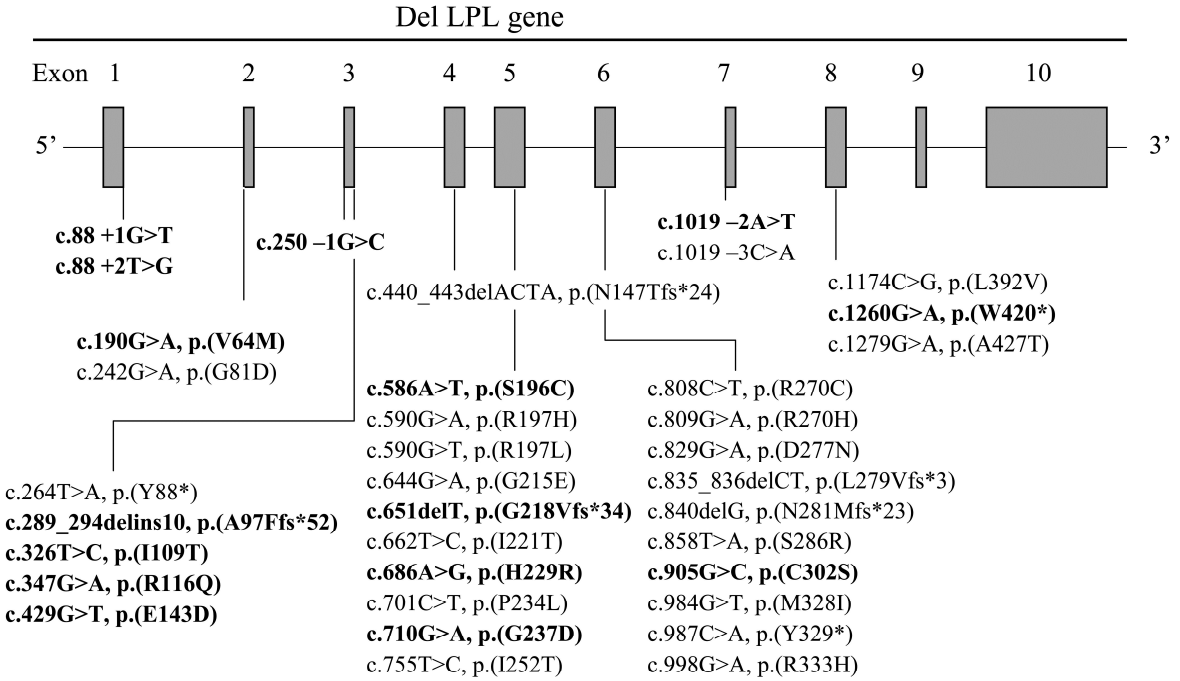

Figure 1

HIGHLIGHTS

- The *LPL* gene was sequenced in individuals with severe/moderate hypertriglyceridemia
- Rare *LPL* variants were found in 33.5% of subjects with severe hypertriglyceridemia
- 26 patients were homozygotes, 9 compound heterozygotes and 21 simple heterozygotes
- Thirty six rare *LPL* variants were identified, 15 of which not reported previously
- Screening of patients' relatives led to the identification of 44 simple heterozygotes

SUPPLEMENTAL MATERIAL

Table S.1. Plasma lipid in homozygotes/compound heterozygotes <1 and >1 year of age.

	< 1 year (1-10 months) [§]	> 1 year (4-74 years)	P*
N.	11	24	
TC (mmol/L) mean ± SD	13.3 ± 7.0	6.8 ± 1.9	0.007
HDL-C (mmol/L) mean ± SD	0.44 ± 0.17	0.59 ± 0.21	NS
TG (mmol/L) median, IQ range	59.7 (29.7-109.5)	23.9 (18.4-35.1)	0.001
Percent with PC or RPC	1/11 (9.0%)	18/24 (75.0%)	0.001

*Mann-Whitney test, Fisher exact test. PC = pancreatitis, RPC = recurrent pancreatitis. [§]Brest-fed or bottle fed-infants.

LPL (8p22) mutations

LPL gene (GenBank-NCBI accession no.): NG_008855.1, GI 210032137, ENSG00000175445

LPL mRNA (GenBank-NCBI accession no.): NM_000237.2, GI 145275217, ENST00000311322

LPL protein (GenBank-NCBI accession no.): NP_000228.1, GI 4557727, ENSP00000309757, UniPro P06858; signal peptide: 27 amino acids.

LPL MUTATIONS FOUND IN ITALY

Table S.2. Sequence variation in the promoter

Location		Effect	
From putative transcription site (at –188bp from ATG)*	c.DNA (from ATG)		Previously reported: S. Ref.
-95 G>T	-283 G>T	No effect on promoter activity	4
-93 T>G	-281 T>G	Decreases promoter activity by 40-50%	4, 35, 39
114 G>A	-61 G>A	?	NEW

References for reviews are reported in RED

Table S.3. Large Rearrangements (Exon Deletions)

Location	cDNA (from ATG)	Proprotein	Ethnic origin (No. of families, No. of patients and genetic status)	Previously reported: S. Ref.
Ex1_10del	c.1_3377del	p.0	Italian (1, 1CHE, 1HE)	46

Table S.4A. Missense mutations

Exon	cDNA	Proprotein	Ethnic origin (No. of families, No. of patients and genetic status)	Previously reported: S. Ref.
2	c.106 G>A	p.(D36N)	Italian (19, 2HO, 15HE)	1, 2, 9, 26, 35, 36, 38, 39, 48
2	c.182 C>T	p.(A61V)	Italian (2, 2HO, 4HE)	NEW
2	c.190 G>A	p.(V64M)	Italian (1, 1HE)	NEW
2	c.242 G>A	p.(G81D)	Italian (2, 1HO, 2HE)	42
3	c.326 T>C	p.(I109T)	Italian (2, 1HO, 2HE)	NEW
3	c.347 G>A	p.(R116Q)	Italian (1, 2HE)	NEW
3	c.429 G>T	p.(E143D)	Italian (1, 1CHE, 1HE)	NEW
5	c.586 A>T	p.(S196C)	Ecuadorian (1, 1HE)	NEW
5	c.590 G>A	p.(R197H)	Italian (1, 1CHE, 1HE)	26, 38, 39
5	c.590 G>T	p.(R197L)	Italian (4, 2HO, 7HE)	12, 14, 39
5	c.644 G>A	p.(G215E)	Italian (4, 1HO, 2CHE, 4HE), Spanish (1, 1CHE, 1HE), Panamanian (1, 1CHE, 1HE)	1, 2, 9, 11, 16, 17, 22, 27, 34, 36, 38, 39, 43, 48, 49, 51
5	c.662 T>C	p.(I221T)	Belgian (1, 1CHE, 1HE)	1, 2, 9, 16, 21, 22, 34, 36, 37, 39
5	c.686 A>G	p.(H229R)	Italian (1, 1CHE, 1HE)	NEW
5	c.701 C>T	p.(P234L)	Panamanian (1, 1CHE, 1HE)	1, 2, 9, 22, 34, 39, 41, 48

5	c.710 G>A	p.(G237D)	Italian (2, 2HO, 4HE)	NEW
5	c.755 T>C	p.(I252T)	Italian (2, 1CHE, 2HE)	1, 2, 9, 35, 36, 38, 39
6	c.808 C>T	p.(R270C)	Serbian (2, 1HO, 2HE)	1, 2, 9, 16, 34, 38, 39, 48
6	c.809 G>A	p.(R270H)	Italian (5, 2HO, 1CHE, 5HE)	1, 2, 9, 16, 34, 38, 39, 44, 47
6	c.829 G>A	p.(D277N)	Italian (9, 1HO, 1CHE, 9HE), Spanish (1, 1CHE, 1HE)	1, 2, 9, 38, 39, 48
6	c.858 T>A	p.(S286R)	Moroccan (2, 1HO, 2HE)	2, 9, 39
6	c.905 G>C	p.(C302S)	Italian (1, 1CHE, 1HE)	NEW
6	c.953 A>G	p.(N318S)	Italian (13, 13HE)	1, 2, 9, 23, 26, 34, 35, 36, 38, 39, 45, 48
6	c.984 G>T	p.(M328I)	Italian (6, 2HO, 1CHE, 6HE)	36, 39
6	c.998 G>A	p.(R333H)	Italian (1, 1HE)	48
8	c.1174 C>G	p.(L392V)	Italian (1, 1HE)	2, 9, 39
8	c.1279 G>A	p.(A427T)	Italian (1, 1HE)	38, 39

References for reviews are reported in RED

Table S.4B. In silico analysis of missense mutations

Exon	cDNA	Proprotein	PolyPhen-2 Hum Div	Poly-Phen-2 Hum Var	SIFT Human Protein	SIFT Blink	Mutation Testing
2	c.106 G>A	p.(D36N)	Benign	Benign	Tolerated	Tolerated	SNP
2	c.182 C>T	p.(A61V)	Benign	Benign	Tolerated	Tolerated	SNP
2	c.190 G>A	p.(V64M)	Probably	Possibly	Damaging	Not tolerated	SNP
2	c.242 G>A	p.(G81D)	Probably	Probably	Damaging	Not tolerated	Disease causing
3	c.326 T>C	p.(I109T)	Probably	Probably	Damaging	Not tolerated	Disease causing
3	c.347 G>A	p.(R116Q)	Probably	Probably	Damaging	Tolerated	Disease causing
3	c.429 G>T	p.(E143D)	Benign	Benign	Damaging	Tolerated	SNP
5	c.586 A>T	p.(S196C)	Probably	Probably	Damaging	Tolerated	SNP
5	c.590 G>A	p.(R197H)	Probably	Probably	Damaging	Not tolerated	SNP
5	c.590 G>T	p.(R197L)	Probably	Possibly	Tolerated	Not tolerated	SNP
5	c.644 G>A	p.(G215E)	Probably	Probably	Tolerated	Tolerated	Disease causing
5	c.662 T>C	p.(I221T)	Probably	Probably	Damaging	Not tolerated	Disease causing
5	c.686 A>G	p.(H229R)	Probably	Probably	Damaging	Not tolerated	Disease causing
5	c.701 C>T	p.(P234L)	Probably	Probably	Damaging	Not tolerated	Disease causing

5	c.710 G>A	p.(G237D)	Probably	Probably	Damaging	Not tolerated	Disease causing
5	c.755 T>C	p.(I252T)	Possibly	Possibly	Damaging	Not tolerated	Disease causing
6	c.808 C>T	p.(R270C)	Possibly	Possibly	Damaging	Not tolerated	Disease causing
6	c.809 G>A	p.(R270H)	Probably	Probably	Damaging	Not tolerated	Disease causing
6	c.829 G>A	p.(D277N)	Possibly	Benign	Damaging	Not tolerated	Disease causing
6	c.858 T>A	p.(S286R)	Possibly	Possibly	Damaging	Tolerated	Disease causing
6	c.905 G>C	p.(C302S)	Benign	Benign	Damaging	Not tolerated	Disease causing
6	c.953 A>G	p.(N318S)	Benign	Benign	Tolerated	Tolerated	Disease causing
6	c.984 G>T	p.(M328I)	Benign	Benign	Damaging	Tolerated	Disease causing
6	c.998 G>A	p.(R333H)	Benign	Benign	Damaging	Tolerated	Disease causing
8	c.1174 C>G	p.(L392V)	Probably	Probably	Damaging	Tolerated	Disease causing
8	c.1279 G>A	p.(A427T)	Benign	Benign	Tolerated	Tolerated	SNP

Table S.4B (continuation). In silico analysis of missense mutations

Exon	cDNA	Proprotein	SNPs3D	SNAP	Overall prediction of pathogenicity
2	c.106 G>A	p.(D36N)	Non-deleterious	Neutral	Non pathogenic
2	c.182 C>T	p.(A61V)	Non-deleterious	Neutral	**Non pathogenic**

2	c.190 G>A	p.(V64M)	Deleterious	Neutral	**4/7 Probably pathogenic**
2	c.242 G>A	p.(G81D)	Deleterious	Non-neutral	**7/7 Pathogenic**
3	c.326 T>C	p.(I109T)	Deleterious	Non-neutral	**7/7 Pathogenic**
3	c.347 G>A	p.(R116Q)	Non-deleterious	Neutral	**4/7 Probably pathogenic**
3	c.429 G>T	p.(E143D)	Non-deleterious	Neutral	**Non pathogenic**
5	c.586 A>T	p.(S196C)	Non-deleterious	Neutral	**3/7 Possibly pathogenic**
5	c.590 G>A	p.(R197H)	Deleterious	Non-neutral	6/7 Pathogenic
5	c.590 G>T	p.(R197L)	Deleterious	Non-neutral	5/7 pathogenic
5	c.644 G>A	p.(G215E)	Non-deleterious	Neutral	3/7 Possibly pathogenic
5	c.662 T>C	p.(I221T)	Deleterious	Non-neutral	7/7 Pathogenic
5	c.686 A>G	p.(H229R)	Deleterious	Non-neutral	**7/7 Pathogenic**
5	c.701 C>T	p.(P234L)	Deleterious	Non-neutral	7/7 Pathogenic
5	c.710 G>A	p.(G237D)	Deleterious	Non-neutral	**7/7 Pathogenic**
5	c.755 T>C	p.(I252T)	Non-deleterious	Non-neutral	6/7 Pathogenic
6	c.808 C>T	p.(R270C)	Deleterious	Non-neutral	7/7 Pathogenic
6	c.809 G>A	p.(R270H)	Deleterious	Non-neutral	7/7 Pathogenic

6	c.829 G>A	p.(D277N)	Deleterious	Non-neutral	6/7 Pathogenic
6	c.858 T>A	p.(S286R)	Deleterious	Non-neutral	6/7 Pathogenic
6	c.905 G>C	p.(C302S)	Deleterious	Non-neutral	**5/7 Probably pathogenic**
6	c.953 A>G	p.(N318S)	Non-deleterious	Neutral	Non pathogenic
6	c.984 G>T	p.(M328I)	Non-deleterious	Non-neutral	3/7 Possibly pathogenic
6	c.998 G>A	p.(R333H)	Non-deleterious	Neutral	2/7 Pathogenic [48]
8	c.1174 C>G	p.(L392V)	Deleterious	Non-neutral	6/7 Pathogenic
8	c.1279 G>A	p.(A427T)	Non-deleterious	Neutral	Non pathogenic

The in silico prediction for novel missense mutations is reported in bold characters.

Table S.4C. Residue conservation during evolution

	Position																							
	36	61	64	81	109	116	143	196	197	215	221	229	234	237	252	270	277	286	302	318	328	333	392	427
Homo sapiens	D	A	V	G	I	R	E	S	R	G	I	H	P	G	I	R	D	S	C	N	M	R	L	A
Baboon	D	A	V	G	I	R	E	S	R	G	I	H	P	G	I	R	D	S	C	N	M	R	L	A
Chimp	D	A	V	G	I	R	E	S	R	G	I	H	P	G	I	R	D	S	C	N	M	R	L	A
Bos taurus	D	T	V	G	I	R	A	S	R	G	I	H	P	G	I	R	D	S	C	N	M	R	L	D

Sus scrofa	D	T	V	G	I	R	A	S	R	G	I	H	P	G	I	R	D	S	C	N	M	R	L	A
Horse	D	T	V	G	I	R	A	S	R	G	I	H	P	G	I	R	D	S	C	N	M	R	L	T
Sheep	D	T	V	G	I	R	A	S	R	G	I	H	P	G	I	R	D	S	C	N	M	R	L	D
Guinea pig	D	T	V	G	I	R	E	S	R	G	I	H	P	G	I	R	D	S	C	N	M	R	L	T
Cat	D	T	V	G	I	R	A	S	R	G	I	H	P	G	I	R	D	S	C	N	M	R	L	T
Mouse	D	A	V	G	I	R	E	S	R	G	I	H	P	G	I	R	D	S	C	N	M	R	L	V
Rat	D	A	V	G	I	R	E	S	R	G	I	H	P	G	I	R	D	S	C	N	M	R	L	V
Chicken	G	M	L	G	I	R	E	I	R	G	I	H	P	G	I	R	D	S	C	N	M	R	L	T
Frog	S	E	V	G	I	R	D	I	I	G	I	H	P	G	I	R	D	S	C	N	M	R	L	N

Table S.4D. Amino acid conservation during evolution, changes in polarity, molecular weight and hydropathy index

Exon	cDNA	Proprotein	Conservation across species	Amino acid substitution		
				Structure	MW	Hydropathy index†
2	c.106 G>A	p.(D36N)	11/13	Charged(-)-polar > Uncharged polar	133.11 > 132.12	-3.5 > -3.5
2	c.182 C>T	p.(A61V)	5/13	Nonpolar > Nonpolar	89.10 > 117.15	1.8 > 4.2
2	c.190 G>A	p.(V64M)	12/13	Nonpolar > Nonpolar	117.15 > 149.21	4.2 > 1.9

2	c.242 G>A	p.(G81D)	13/13	Nonpolar > Charged(-)-polar	75.07 > 133.11	-0.4 > -3.5
3	c.326 T>C	p.(I109T)	13/13	Nonpolar > Uncharged polar	131.18 > 119.12	4.5 > -0.7
3	c.347 G>A	p.(R116Q)	13/13	Charged(+)-polar > Uncharged polar	174.20 > 146.15	-4.5 > -3.5
3	c.429 G>T	p.(E143D)	7/13	Charged(-)-polar > Charged(-)-polar	147.13 > 133.11	-3.5 > -3.5
5	c.586 A>T	p.(S196C)	11/13	Uncharged polar > Uncharged polar	105.09 > 121.16	-0.8 > 2.5
5	c.590 G>A	p.(R197H)	12/13	Charged(+)-polar > Charged(+)-polar	174.20 > 155.16	-4.5 > -3.2
5	c.590 G>T	p.(R197L)	12/13	Charged(+)-polar > Nonpolar	174.20 > 131.18	-4.5 > 3.8
5	c.644 G>A	p.(G215E)	13/13	Nonpolar > Charged(-)-polar	75.07 > 147.13	-0.4 > -3.5
5	c.662 T>C	p.(I221T)	13/13	Nonpolar > Uncharged polar	131.18 > 119.12	4.5 > -0.7
5	c.686 A>G	p.(H229R)	13/13	Charged(+)-polar > Charged(+)-polar	155.15 > 174.20	-3.2 > -4.5
5	c.701 C>T	p.(P234L)	13/13	Nonpolar > Nonpolar	115.13 > 131.18	-1,6 > 3.8
5	c.710 G>A	p.(G237D)	13/13	Nonpolar > Charged(-)-polar	75.07 > 133.11	-0.4 > -3.5
5	c.755 T>C	p.(I252T)	13/13	Nonpolar > Uncharged polar	131.18 > 119.12	4.5 > -0.7
6	c.808 C>T	p.(R270C)	13/13	Charged(+)-polar > Uncharged polar	174.20 > 121.16	-4.5 > 2.5
6	c.809 G>A	p.(R270H)	13/13	Charged(+)-polar > Charged(+)-polar	174.20 > 155.15	-4.5 > -3.2
6	c.829 G>A	p.(D277N)	13/13	Charged(-)-polar > Uncharged polar	133.11 > 132.12	-3.5 > -3.5

6	c.858 T>A	p.(S286R)	13/13	Uncharged polar > Charged(+)-polar	105.09 > 174.20	-0.8 > -4.5
6	c.905 G>C	p.(C302S)	13/13	Uncharged polar > Uncharged polar	121.16 > 105.09	2.5 > -0.8
6	c.953 A>G	p.(N318S)	13/13	Uncharged polar > Uncharged polar	132.12 > 105.09	-3.5 > -0.8
6	c.984 G>T	p.(M328I)	13/13	Nonpolar > Nonpolar	149.21 > 131.18	1.9 > 4.5
6	c.998 G>A	p.(R333H)	13/13	Charged(+)-polar > Charged(+)-polar	174.20 > 155.15	-4.5 > -3.2
8	c.1174 C>G	p.(L392V)	13/13	Nonpolar > Nonpolar	131.18 > 117.15	3.8 > 4.2
8	c.1279 G>A	p.(A427T)	4/13	Nonpolar > Uncharged polar	89.10 > 119.12	1.8 > -0.7

[†]Kyte J and Doolittle RF. A simple method for displaying the hydropathic character of a protein. J Mol Biol 1982; 157: 105-132.

Table S.5. Nonsense mutations

Exon	cDNA	Proprotein	Ethnic origin (No. of families, No. of patients and genetic status)	Previously reported: S. Ref.
3	c.264 T>A	p.(Y88*)	Belgian (1, 1CHE, 1HE)	1, 2, 9, 16, 39
6	c.987 C>A	p.(Y329*)	Italian (4, 3HO, 9HE)	8, 9, 39
8	c.1260 G>A	p.(W420*)	Italian (2, 1HO, 2HE)	NEW
9	c.1421 C>G	p.(S474*)	Italian (3, 3HE)	1, 2, 9, 11, 16, 30, 35, 36, 38, 39, 48

References for reviews are reported in RED

Table S.6. Insertions/deletions of a single or few nucleotides

Exon	cDNA	Proprotein	Ethnic origin (No. of families, No. of patients and genetic status)	Previously reported: S. Ref.
3	c.289-294delGCCGCC insTTTGCCAAAA	p.(A97Ffs*52)	Pakistani (2, 1HO, 2HE)	NEW
4	c.440-443delACTA	p.(N147Tfs*24)	Italian (1, 1HE)	2, 9, 36, 39
5	c.651delT	p.(G218Vfs*34)	Italian (3, 1HO, 3HE)	NEW
6	c.835-836delCT	p.(L279Vfs*3)	Italian (2, 2HO, 2HE)	9, 39
6	c.840delG	p.(N281Mfs*23)	Italian (1, 1CHE, 1HE)	46

References for reviews are reported in RED

Table S.7. Splicing mutations

cDNA	mRNA analysis[†] *in silico* analysis[‡]	Proprotein From mRNA analysis[†] Predicted *in silico*[‡]	Ethnic origin (No. of families, No. of patients and genetic status)	Previously reported: S. Ref.
IVS1 c.88 +1G>T	c.88+1_418ins[‡]	p.(Q30Rfs*45)[‡]	Italian (1, 1CHE, 1HE)	NEW
IVS1 c.88 +2T>G	c.88+1_418ins[‡]	p.(Q30Rfs*45)[‡]	Italian (2, 1CHE, 2HE)	NEW
IVS2 c.250 –1G>C	r.[250-1g>c; 250-72_250-1ins, 250_256del][†]	p.[(T85Yfs*15, V84Efs*86)][†]	Italian (2, 1HO, 2HE)	NEW

IVS6 c.1019 –2A>T	r.1019_1139del[†]	p.(V340Gfs*13)[†]	Italian (2, 2HO, 2HE)	NEW
IVS6 c.1019 –3C>A	r.[1019_1139del, 776_1427del][†]	p.[(V340Gfs*13, D259Efs*13)][†]	Tunisian (2, 1HO, 2HE)	9, 39

DS: donor splice site. AS: acceptor splice site. [†] documented by mRNA analysis; [‡] Overall prediction from *in silico* analysis performed with Human Splicing Finder, NetGene2 and Automated Splice Site Analyses. References for reviews are reported in RED

Table S.8. Hypertriglyceridemic subjects carrying common *LPL* variants

	Subjects with severe HTG (TG >10 mmol/L)	Subjects with moderate HTG (TG >4.5<10 mmol/L)
LPL variants	n. 5 HE p.(D36N), n. 1 HO p.(D36N), n. 1 HE p.(N318S)	n. 9 HE p.(D36N), n. 1 HO p.(D36N), n. 10 HE p.(N318S)
M/F	3/4	18/2
Age (years)	33.1 ± 10.5	42.4 ± 13.4
BMI (kg/m^2)	25.5 ± 4.5	25.4 ± 3.1
Total cholesterol (mmol/L)	7.09 ± 0.96	6.20 ± 0.96
HDL cholesterol (mmol/L)	0.75 ± 0.12	0.76 ± 0.15
Triglycerides (mmol/L)	15.9 ± 5.7	7.0 ± 1.5

Table S.9. Previously reported rare and common *LPL* variants identified in patients with severe or moderate hypertriglyceridemia.

Suppl. References	Wang J et al. 2007 [22]	Wright WT et al. 2008 [26]	Evans D et al. 2011 [36]			Surendran RP et al. 2012 [38]	Martin-Campos et al. 2014 [48]
Subjects screened	n. 110 with TG >10mmol/L	n. 19 with TG >14 mmol/L	n. 107 with TG >10 mmol/L	n. 206 with TG <10 mmol/L	n. 109 with Type III	n. 86 with TG > 10 mmol/L	n. 29 with chylomicronemia
Age (years)	49.9 ± 12.9	50.0 ± 12.0	46.4 ± 10.8	44.4 ± 12.5	49.3 ± 11.3	41 (1-69)	Newborn n. 9 Children n. 9 Adolescents n. 2 Adults n. 9
HO	-	n. 1 p.(D36N)	-	-	-	n. 1 p.(G161E)	n. 2 p.(D36N)
„	-	-	-	-	-	n.1 p.(G166S)	n. 2 p.(D177N)
„	-	-	-	-	-	n. 3 p.(G181S)	n. 3 p.(G215E)
“	-	-	-	-	-	n. 5 p.(D183G)	n. 1 p.(P234L)
“	-	-	-	-	-	n. 1 p.(P184R)	-
“	-	-	-	-	-	n. 2 p.(N318S)	-
“	-	-	-	-	-	n. 2 p.(L380Afs*2)	-
CHE	-	n. 1 p.(D36N) + p.(N318S)	n. 1 p.(D36N) + p.(Y289*)	n. 1 p.(S293P) + p.(N318S)	n. 1 p.(I252T) + p.(S474*)	n. 2 p.(V96L) + p.(G215E)	n. 1 p.(Q16Efs*23) + p.(G215E)
“	-	n. 1 p.(N318S) + p.(V340I)	n. 1 p.(V96L) + p.(G215E)	-	-	n. 1 p.(V96L) + p.(R270H)	n. 1 p.(D36N) + p.(G215E)

“	-	-	n. 1 p.(V96L) + p.(N318S)	-	-	n. 1 p.(I252T) + p.(R270H)	n. 1 p.(D36N) + p.(S474*)
“	-	-	n. 1 p.(I252T) + p.(S474*)	-	-	n. 1 p.(D277N) + p.(S278C)	n. 1 p.(W113G) + p.(W421*)
“	-	-	-	-	-	-	n.1 p.(A125Gfs*22) + p.(H273R)
“	-	-	-	-	-	-	n. 1 p.(G215E) + p.(P234L)
“	-	-	-	-	-	-	n. 1 p.(G215E) + p.(R333H)
“	-	-	-	-	-	-	n. 1 p.(G215E) + p.(D277N)
“	-	-	-	-	-	-	n. 1 p.(P234L) + p.(H273R)
HE	n. 1 p.(Q16Efs*24)	n. 1 p.(R197H)	n. 1 p.(M1L)	n. 2 p.(V96L)	n. 1 p.(E374D)	n. 6 p.(D36N)	n. 1 p.(T85Kfs*13)
“	n. 12 p.(D36N)	n. 4 p.(N318S)	n. 1 p.(V96L)	n. 1 p.(T44Nfs*3)	-	n. 1 p.(V96L)	n. 1 p.(R270C)
“	n. 1 p.(D52H)	-	n. 1 p.(I221T)	n. 1 p.(R116W)	-	n. 2 p.(R197H)	n. 1 p.(S474*)
“	n. 1 p.(W113R)	-	n. 1 p.(K129Sfs*17)	n. 1 p.(Y121S)	-	n. 1 p.(V206A)	-
“	n. 2 p.(G215E)	-	n. 1 p.(N147Tfs*24)	n. 1 p.(G215E)	-	n. 1 p.(G215E)	-
“	n. 1 p.(I221T)	-	n. 1 p.(G215E)	n. 1 p.(I223F)	-	n. 1 p.(L301Sfs*3)	-

“	n. 1 p.(P234L)	-	n. 1 p.(S304N)	n. 1 p.(Y233D)	-	n. 10 p.(N318S)	-
“	n. 4 p.(N318S)	-	n. 1 p.(M328I)	n.1 p.(C305Y)	-	n. 1 p.(H348Qfs*43)	-
“	n. 7 p.(S474*)	-	n. 1 p.(T379I)	-	-	n. 2 p.(T379I)	-
“	-	-	-	-	-	n. 2 p.(A427T)	-

Analysis of splice site mutations of *LPL* gene.

c.250 -1G>C (Intron 2)

Construction of the reporter minigene: a 3767 nt fragment of wild type LPL gene, containing exon 2, intron 2 and exon 3 was inserted in pTargeT plasmid vector. The splice site mutation was introduced by site directed mutagenesis. The wild type and mutant plasmid were transfected in COS1 cells. The wild type minigene generated a transcript of 340 nt, while the mutant minigene generated two transcripts of 411 nt and 332 nt, respectively. In the 332 nt transcript exon 2 joined to exon 3 devoid of the first 7 nt at the 5’ end. In the 441 fragment exon 2 was followed by 72 nucleotides 3’ of intron 2 (partial intron retention) and by exon 3. The resulting frameshifts led to the insertion of a premutare termination codon. The predicted translation products of the mutant transcript are two truncated proteins p.[(T85Yfs*15, V84Efs*86] expected to be devoid of function.

c.1019 -2A>T (Intron 6)

Construction of the reporter minigene: a 1311 nt fragment of wild type LPL gene spanning from intron 6 to intron 8 was obtained by PCR amplification. To reduce the size of the minigene, intron 6 and intron 7 had been shortened by producing an internal deletion of 3.5 kb and 7.5 kb, respectively. The genomic DNA fragment was inserted in a pTargeT vector and the splice site mutation was introduced by site directed mutagenesis.

The wild type and mutant plasmid were transfected in COS1 cells. The wild type minigene generated a 508 nt transcript while the mutant minigene generated a 347 nt transcript. The sequence of the 347 transcripts showed that exon 6 joined directly to exon 8 with the complete skipping of exon 7. This leads to a frameshift with the formation of a premature termination codon. The product of this transcript is predicted to be a truncated protein p.(V340Gfs*13) devoid of function.

Table S.10. LPL mutations described in literature

Promoter			
Location		Effect	
From putative transcription site (at –188bp from ATG)*	cDNA (from ATG)		Ref.
- 95 G>T	- 283 G>T	No effect on promoter activity	4
- 93 T>G	-281 T>G	Decreases promoter activity by 40-50%	4, 35, 39
- 79 T>G	- 267 T>G	No effect on promoter activity	4
-53 G>C	-241 G>C	Decreases promoter activity by 70-75%	4, 39
-39 T>C	-227 T>C	Decreases promoter activity by 85%	4, 39
+18 ins CC (TCCCCCinsCCTC)	-170 ins CC	Decreases promoter activity by 20-50%	4, 39

*****More recently located at –370bp from ATG; References for reviews are reported in RED

Major rearrangements			

Exon (kb)	cDNA	Proprotein	Ref.
Promoter-Ex1 del (54kb)	c.1-?_88+?del	p.0	39
Ex1_10del	c.1_3377del	p.0	46
Ex2 del (2.3kb del, 150nt ins)	c.89-?_249+? del	p.(Q30Rfs*6) or p.0	24, 39
Ex3-5 del (6kb)	c.250-?_775+? del	p.0	1, 2, 9, 39
Ex6 partial (2kb dup)	c.897-?_1018+?dup	p.0	1, 2, 9, 39
Ex8-10 del	c.1140- ?_1428+?del	p.0	33, 39
Ex9 del (2.1kb)	c.1323-?_1427+?del	p.0	1, 2, 9

References for reviews are reported in RED

Missense mutations				
Exon	cDNA	Codon	Proprotein	Ref.
1	c.1 A>C	ATG>CTG	p.(M1L)	36, 39
1	c.3 G>C	ATG>ATC	p.(M1I)	19, 39
2	c.106 G>A	GAC>AAC	p.(D36N)	1, 2, 9, 26, 35, 36, 38, 39, 48
2	c.113 A>G	GAA>GGA	p.(E38G)	34, 39
2	c.143 A>T	GAC>GTC	p.(D48V)	2

2	c.154 G>C	GAC>CAC	p.(D52H)	22, 34, 39
2	c.209 A>G	AAT>AGT	p.(N70S)	2, 9, 16, 39
2	c.211 C>T	CAC>TAC	p.(H71Y)	2
2	c.213 C>G	CAC>CAG	p.(H71Q)	39
2	c.214 A>G	AGC>GGC	p.(S72G)	25, 39
2	c.242 G>A	GGC>GAC	p.(G81D)	42
3	c.286 G>C	GTG>CTG	p.(V96L)	1, 2, 9, 33, 34, 35, 36, 38, 39
3	c.292 G>A	GCC>ACC	p.(A98T)	10, 11, 23, 39, 50
3	c.306 A>T	AGA>AGT	p.(R102S)	2, 9, 39
3	c.337 T>C	TGG>CGG	p.(W113R)	1, 2, 9, 22, 29, 34, 39
3	c.337 T>G	TGG>GGG	p.(W113G)	9, 39, 48
3	c.346 C>T	CGG>TGG	p.(R116W)	36, 39
3	c.362 A>C	TAC>TCC	p.(Y121S)	36, 39
3	c.373 G>A	GCG>ACG	p.(A125T)	9, 39
3	c.382 A>G	ACC>GCC	p.(T128A)	9, 39
3	c.394 G>A	GGA>AGA	p.(G132R)	9, 16, 39

4	c.464 T>C	CTC>CCC	p.(L155P)	49
4	c.482 G>A	GGA>GAA	p.(G161E)	38
4	c.488 A>G	CAT>CGT	p.(H163R)	1, 2, 9, 39
4	c.496 G>A	GGC>AGC	p.(G166S)	1, 2, 9, 38, 39
4	c.506 G>A	GGA>GAA	p.(G169E)	1, 2, 9, 39
5	c.541 G>A	GGC>AGC	p.(G181S)	1, 2, 9, 38, 39
5	c.541 G>C	GGC>CGC	p.(G181R)	43
5	c.542 G>T	GGC>GTC	p.(G181V)	9, 16, 39
5	c.547 G>A	GAT>AAT	p.(D183N)	1, 2, 9, 39
5	c.547 G>C	GAT>CAT	p.(D183H)	2, 9, 39
5	c.548 A>G	GAT>GGT	p.(D183G)	1, 2, 9, 38, 39
5	c.551 C>G	CCA>CGA	p.(P184R)	1, 2, 9, 38, 39
5	c.553 G>A	GCT>ACT	p.(A185T)	9, 39
5	c.557 G>A	GGA>GAA	p.(G186E)	28, 39
5	c.569 A>G	GAG>GGG	p.(E190G)	9, 39
5	c.570 G>T/C	GAG> GA(T/C)	p.(E190D)	2

5	c.590 G>T	CGT>CTT	p.(R197L)	12, 14, 39
5	c.590 G>A	CGT>CAT	p.(R197H)	26, 38, 39
5	c.596 C>G	TCT>TGT	p.(S199C)	1, 2, 9, 39
5	c.602 A>T	GAT>GTT	p.(D201V)	15, 39
5	c.607 G>A	GCA>ACA	p.(A203T)	1, 2, 9, 39
5	c.617 T>C	GTA>GCA	p.(V206A)	38
5	c.621 C>G	GAC>GAG	p.(D207E)	2, 9, 39
5	c.622 G>A	GTC>ATC	p.(V208I)	10, 11, 39
5	c.628 C>G	CAC>GAC	p.(H210D)	9, 39
5	c.630 C>G	CAC>CAG	p.(H210Q)	2, 9, 39
5	c.637 A>G	ACC>GCC	p.(T213A)	34, 39
5	c.643 G>A	GGG>AGG	p.(G215R)	2, 9, 39
5	c.644 G>A	GGG>GAG	p.(G215E)	1, 2, 9, 11, 16, 17, 22, 27, 34, 36, 38, 39, 43, 48, 49, 51
5	c.656 G>A	CGA>CAA	p.(R219Q)	39
5	c.658 A>C	AGC>CGC	p.(S220R)	9, 21, 39
5	c.662 T>C	ATT>ACT	p.(I221T)	1, 2, 9, 16, 21, 22, 34, 36, 37, 39

5	c.665 G>A	GGA>GAA	p.(G222E)	2, 9, 39
5	c.667 A>T	ATC>TTC	p.(I223F)	36, 39
5	c.674 A>G	AAA>AGA	p.(K225R)	16, 39
5	c.680 T>C	GTT>GCT	p.(V227A)	16, 39
5	c.680 T>G	GTT>GGT	p.(V227G)	52
5	c.693 C>G	GAC>GAG	p.(D231E)	1, 2, 9, 16, 39
5	c.695 T>G	ATT>AGT	p.(I232S)	1, 2, 9, 39
5	c.697 T>G	TAC>GAC	p.(Y233D)	36, 39
5	c.701 C>T	CCG>CTG	p.(P234L)	1, 2, 9, 22, 34, 39, 41, 48
5	c.721 C>T	CCA>TCA	p.(P241S)	32, 51
5	c.722 C>T	CCA>CTA	p.(P241L)	6
5	c.727 T>A	TGT>AGT	p.(C243S)	1, 2, 9, 39
5	c.755 T>C	ATT>ACT	p.(I252T)	1, 2, 9, 35, 36, 38, 39
5	c.755 T>A	ATT>AAT	p.(I252N)	30
6	c.798 C>G	TGC>TGG	p.(C266W)	9, 39
6	c.805 G>A	GAG>AAG	p.(E269K)	9, 11, 19, 39

6	c.808 C>T	CGC>TGC	p.(R270C)	1, 2, 9, 16, 34, 38, 39, 48
6	c.809 G>A	CGC>CAC	p.(R270H)	1, 2, 9, 16, 34, 38, 39, 44, 47
6	c.809 G>T	CGC>CTC	p.(R270L)	2, 39
6	c.811 T>A	TCC>ACC	p.(S271T)	1, 2, 9, 39
7	c.818 A>G	CAT>CGT	p.(H273R)	48
6	c.826 A>G	ATC>GTC	p.(I276V)	34, 39
6	c.827 T>C	ATC>ACC	p.(I276T)	9, 39
6	c.829 G>A	GAC>AAC	p.(D277N)	1, 2, 9, 38, 39, 48
6	c.833 C>G	TCT>TGT	p.(S278C)	1, 2, 9, 38, 39
6	c.833 C>T	TCT>TTT	p.(S278F)	39
6	c.835 C>G	CTG>GTG	p.(L279V)	9, 10, 20, 21, 39, 50
6	c.836 T>G	CTG>CGG	p.(L279R)	2, 9, 10, 21, 39
6	c.856 A>G	AGT>GGT	p.(S286G)	9, 39
6	c.858 T>A	AGT>AGA	p.(S286R)	2, 9, 39
6	c.862 G>A	GCC>ACC	p.(A288T)	1, 2, 9, 16, 39
6	c.865 T>C	TAC>CAC	p.(Y289H)	1, 2, 9, 39

6	c.872 G>A	TGC>TAC	p.(C291Y)	7, 39
6	c.877 T>C	TCC>CCC	p.(S293P)	2, 36, 39
6	c.891 T>G	TTT>TTG	p.(F297L)	9, 16, 39
6	c.905 G>T	TGC>TTC	p.(C302F)	34, 39
6	c.909 G>C	TTG>TTC	p.(L303F)	13, 16, 39
6	c.911 G>A	AGT>AAT	p.(S304N)	36, 39
6	c.913 T>C	TGT>CGT	p.(C305R)	16, 25, 39
6	c.914 G>A	TGT>TAT	p.(C305Y)	36, 39
6	c.928 T>A	TGC>AGC	p.(C310S)	39
6	c.929 G>A	TGC>TAC	p.(C310Y)	10, 11, 39
6	c.938 T>C	CTG>CCG	p.(L313P)	2, 9, 23, 39
6	c.953 A>G	AAT>AGT	p.(N318S)	1, 2, 9, 23, 26, 34, 35, 36, 38, 39, 45, 48
6	c.975 C>G	AGC>AGG	p.(S325R)	10, 11, 39
6	c.983 T>C	ATG>ACG	p.(M328T)	9, 39
6	c.983 T>G	ATG>AGG	p.(M328R)	17, 39
6	c.984 G>T	ATG>ATT	p.(M328I)	36, 39

6	c.989 T>C	CTG>CCG	p.(L330P)	9, 39
3	c.998 G>A	CGT>CAT	p.(R333H)	48
6	c.1018 G>A	GTC>ATC	p.(V340I)	26, 39
7	c.1033 G>A	GTA>ATA	p.(V345I)	34, 39
7	c.1049 C>G	TCT>TGT	p.(S350C)	9, 39
7	c.1051 G>A	GGG>AGG	p.(G351R)	51
7	c.1081 G>A	GCC>ACC	p.(A361T)	2, 9, 16, 39
7	c.1094 C>T	TCT>TTT	p.(S365F)	10, 11, 39
7	c.1108 G>A	GTG>ATG	p.(V370M)	39
7	c.1122 C>G	GAG>GAC	p.(E374D)	36, 39
7	c.1134 C>G	TTC>TTG	p.(F378L)	5, 39
7	c.1135 A>G	ACT>GCT	p.(T379A)	39
7	c.1136 C>T	ACT>ATT	p.(T379I)	2, 36, 38, 39
8	c.1174 C>G	CTA>GTA	p.(L392V)	2, 9, 39
8	c.1279 G>A	GCC>ACC	p.(A427T)	38, 39
8	c.1302 A>T	AAA>AAT	p.(K434N)	53

8	c.1306 G>A	GGA>AGA	p.(G436R)	53
8	c.1309 G>A	GAG>AAG	p.(E437K)	9, 39
8	c.1310 A>T	GAG>GTG	p.(E437V)	2, 9, 39, 40
9	c.1334 G>A	TGT>TAT	p.(C445Y)	9, 37, 39, 40
9	c.1342 G>A	GAG>AAG	p.(E448K)	9, 37, 39, 40

References for reviews are reported in RED

Nonsense mutations				
Exon	cDNA	Codon	Proprotein	Ref.
1	c.41 G>A	TGG>TAG	p.(W14*)	9, 39
1	c.42 G>A	TGG>TGA	p.(W14*)	3, 16
2	c.162 C>A	TGC>TGA	p.(C54*)	20, 39
3	c.264 T>A	TAT>TAA	p.(Y88*)	1, 2, 9, 16, 39
3	c.272 G>A	TGG>TAG	p.(W91*)	1, 2, 9, 39
3	c.300 C>A	TAC>TAA	p.(Y100*)	2, 9, 39
3	c.397 C>T	CAG>TAG	p.(Q133*)	1, 2, 9, 39
5	c.655 C>T	CGA>TGA	p.(R219*)	5, 39

6	c.798 C>A	TGC>TGA	p.(C266*)	2, 9, 16, 39
6	c.867 C>A	TAC>TAA	p.(Y289*)	1, 2, 9, 36, 39
6	c.867 C>G	TAC>TAG	p.(Y289*)	1, 9
6	c.873 C>A	TGC>TGA	p.(C291*)	9, 39
6	c.945 T>A/G	TAT>TAA/G	p.(Y315*)	9, 39
6	c.987 C>A	TAC>TAA	p.(Y329*)	8, 9, 39
8	c.1226 G>A	TGG>TAG	p.(W409*)	2, 16, 39
8	c.1227 G>A	TGG>TGA	p.(W409*)	2, 9
8	c.1262 G>A	TGG>TAG	p.(W421*)	27, 39, 48
9	c.1421 C>G	TCA>TGA	p.(S474*)	1, 2, 9, 11, 16, 30, 35, 36, 38, 39, 48

References for reviews are reported in RED

Insertions/deletions of a single or few nucleotides			
Exon	cDNA	Proprotein	Ref.
1	c.46_47delCA	p.(Q16Efs*24)	22, 34, 39, 48
2	c.128dupT	p.(R44Kfs*4)	45
2	c.133dupA	p.(T44Nfs*3)	36

2	c.133_143delACCCCTGAAGA	p.(D48Hfs*3)	9, 39
2	c.183dupA	p.(E62Rfs*28)	9, 39
2/IVS2	c.247_249+1delACGg	p.(T83*)	28
3	c.286_287delGT	p.(V96Gfs*51)	9, 39
3	c.290_293delCCGCinsGG	p.(A97Gfs*50)	9, 39
3	c.373_374insG	p.(A125Gfs*23)	48
3	c.384delCinsTGGGCT	p.(K129Gfs*45)	1, 2, 9, 39
3	c.386_390delAACTG	p.(K129Sfs*17)	35, 36
4	c.440_443delACTA	p.(N147Tfs*24)	2, 9, 36, 39
5	c.596delC	p.(S199Ffs*8)	18, 39
5	c.708delA	p.(G237Vfs*15)	2
5	c.742delG	p.(A248Lfs*4)	1, 2, 9, 16, 39
5	c.767_768insTAAATATT	p.(L257Kfs*10)	1
6	c.835_836delCT	p.(L279Vfs*3)	9, 39
6	c.840delG	p.(N281Mfs*23)	46
6	c.901delC	p.(L301Sfs*3)	38

6	c.953delA	p.(N318Ifs*13)	9, 16, 39
6	c.1016_1017insC	p.(K339Nfs*15)	23, 39
7	c.1044_1050delTTTTTCT	p.(H348Qfs*43)	38
7	c.1138_1139delCT	p.(L380Afs*2)	21, 38, 39
8	c.1163-1164insA	p.(Y389Lfs*24)	23, 39
8	c.1227delG	p.(W409*)	1, 16
8	c.1267_1272delAGTCCC	p.(S423_P424del)	9, 39
10	c.1840_1844(*172_176)delTACTC	-	31

References for reviews are reported in RED

Splicing mutations		
cDNA	Effect	Ref.
IVS1 c.88 +1G>C	Loss of DS; new DS in IVS1; p.(Q30Pfs*45) or p.0	2, 9, 39
IVS1 c.88 +2dupT	Weakening of DS; new DS in IVS1; p.(Q30Pfs*45) or p.0	32, 51
IVS1 c.89 –1G>C	Loss of AS, new AS in Ex2 or skipping of Ex2 ; p.(Q30Pfs*6) or p.0	51
IVS1 c.89 –2_4delCCA	r.88_249del, p.(Q30Rfs*6)	9, 39
IVS2 c.249 +1G>A	Loss of DS; new DS in IVS2; c.249_250ins38, p.(T85Kfs*13)	1, 2, 9, 16, 39, 51

IVS2 c.249 +2_3insT	Loss of DS; new DS in IVS2; c.249_250ins38, p.(T85Kfs*13)	48
IVS2 c.250 –1G>A	Loss of AS, new AS in IVS2; c.249_250ins72, p.(T83fs*16)	1, 2, 9, 39
IVS3 c.430 –6C>T	Weakening of AS; new AS in Ex4; c.430_505del, p.(E143fs*3)	2, 3, 9, 39
IVS6 c.1019 –3C>T	Skipping Ex7; c.1019_1139del, p.(V340Gfs*13)	9
IVS6 c.1019 –3C>A	Skipping Ex7; r.1019_1139del, p.(V340Gfs*13) Skipping Ex6_9; r.776_1427del, p.(D259Efs*13)	9, 39
IVS8 c.1322 +2T>C	Loss of DS, new DS in Ex 8; r.1188_1322del, p.(K441Gfs*6)	9, 16, 39

References for reviews are reported in RED

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