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NOVEL MUTATIONS IN THE GPIHBP1 GENE IDENTIFIED IN TWO PATIENTS WITH RECURRENT ACUTE PANCREATITIS

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ABSTRACT

**Background:** Glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1 (GPIHBP1) has been demonstrated to be essential for the *in vivo* function of Lipoprotein Lipase (LPL), the major triglyceride (TG) hydrolyzing enzyme involved in the intravascular lipolysis of TG-rich lipoproteins. Recently loss of function mutations of *GPIHBP1* have been reported as the cause of Type I hyperlipoproteinemia in several patients.

**Methods:** Two unrelated patients were referred to our Lipid Units because of a severe hypertriglyceridemia and recurrent pancreatitis. We measured LPL activity in post-heparin plasma and serum ApoCII and sequenced *LPL, APOC2* and *GPIHBP1*.

**Results:** The two patients exhibited very low LPL activity not associated with mutations in *LPL* gene or with ApoCII deficiency. The sequence of *GPIHBP1* revealed two novel point mutations. One patient (Proband 1) was found to be homozygous for a C>A transversion in exon 2 resulting in the conversion of threonine to lysine at position 80 (p.Thr80Lys). The other patient (Proband 2) was found to be homozygous for a G>T transversion in the third base of the ATG translation initiation codon in exon 1, resulting in the conversion of methionine to isoleucine (p.Met1Ile).

**Conclusion:** In conclusion, we have identified two novel *GPIHBP1* missense mutations in two unrelated patients as the cause of their severe hypertriglyceridemia.

**Keywords:** Glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1 (GPIHBP1), severe hypertriglyceridemia, recurrent pancreatitis, lipoprotein lipase activity.
1. Introduction

Type I and V hyperlipoproteinemia are characterized by high concentrations of chylomicrons in the fasting state, a condition which increases the risk of acute pancreatitis. Monogenic forms of familial hyperchylomicronemia are due to defects in the lipolytic cascade of triglyceride-rich lipoproteins that may result from mutations in at least five different genes: LPL, encoding the enzyme lipoprotein lipase (LPL; OMIM #238600) and involved in the majority of cases of chylomicronemia; APOC2, encoding the Apolipoprotein CII, the activator of LPL (ApoCII; OMIM #207750); APOA5, encoding the Apolipoprotein AV, also an activator of LPL (ApoAV; OMIM #144650); LMF1, encoding the Lipase Maturation Factor 1, a tissue factor which allows the secretion of functional LPL and Hepatic Lipase (HL; OMIM #611761) and GPIHBP1, encoding the Glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1, the molecular platform for LPL on the endothelial surface of capillaries, (GPIHBP1; OMIM #612757).

GPIHBP1 is a 184 aa endothelial cell protein which acts as a transporter for LPL across the endothelial cells to the capillary lumen and appears to be the main binding site for LPL on the endothelial surface. GPIHBP1 belongs to the Ly6 protein family, so called because of a lymphocyte antigen 6 domain that contains 10 cysteine residues forming disulfide bonds and creating a characteristic three-finger structural motif. The Ly6 domain is crucial because it is involved in the binding to LPL and allows the interactions of LPL with ApoCII, ApoAV and TG-rich lipoproteins on the endothelial surface.

Most missense mutations found in GPIHBP1 causing type I hyperlipoproteinemia are located in the Ly6 domain and many of them affect a cysteine residue. Interestingly, it has been recently described that many amino acid substitutions in this domain lead to the formation of disulfide-linked dimers and multimers. The formation of multimers explains the loss of function of GPIHBP1 as GPIHBP1 monomers are capable of binding LPL. In addition, a mutation affecting the C-terminal domain has been shown to impair GPIHBP1 trafficking to the endothelial cell surface. Finally, nonsense/frameshift mutations and large exon deletions in this gene have also been described in some patients with Type I hyperlipoproteinemia.

In this study we report two novel GPIHBP1 missense mutations identified in two unrelated patients as the cause of their severe hypertriglyceridemia.

2. Materials and methods

2.1. Subjects

Clinical, demographic, anthropometric and laboratory data were retrieved from clinical records of two patients who attended the outpatient clinic at Hospital La Paz (Madrid) and Hospital de Reus (Tarragona) respectively. Informed written consent has been obtained from the patients and their relatives participating to the study. The study was approved by the Ethic Committee of the participating institutions.

2.2. Plasma lipid analyses

Serum samples were obtained after an overnight fasting. Cholesterol and triglycerides were determined by automated end-point enzymatic methods. Serum ApoCII was quantified by immunoturbidimetry in a Mindray Bs-380 Clinical Autoanalyzer (Mindray, zhensheng, China) using commercial assays (Spinreact. Barcelona, Spain).
2.3. LPL activity assay

A blood sample was drawn from each proband 15 minutes after the intravenous injection of sodium heparin (100 units/kg) in order to measure post-heparin plasma LPL activity. LPL activity was measured using a lipid emulsion containing triolein [9,10-3H(N)] (Perkin Elmer NET431) as a substrate according to Olivecrona et al.20.

2.4. Genetic analyses

Genomic DNA was isolated from frozen whole blood in EDTA using an EZ1 BioRobot® (QIAGEN, Hilden, Germany) with the appropriate reagents. The genotyping of common polymorphisms in APOE (rs429358, rs7412) and APOA5 (rs3135506, rs662799) was performed using TaqMan assays in a real-time thermal cycler CFX96™ (BioRad, California, USA), the iQ™ Supermix and the allele discrimination mode of the CFX96™ software, as described previously21.

The sequencing of the LPL, APOC2 and GPIHBP1 exons and splice junctions was carried out as previously described19. The mutations were designated according to the Human Genome Variation Society, 2012 version (http://www.hgvs.org/mutnomen/recs-DNA.html). GPIHBP1 protein sequence variants were designated according to http://www.hgvs.org/mutnomen/recs-prot.html.

The biological impact of the novel missense mutations found in the probands were tested in silico with two algorithms: SIFT (Sort Intolerant From Tolerant). sift.jcvi.org and Polyphen 2; genetics.bwh.harvard.edu/pph2/.

The two novel GPIHBP1 mutations found in the probands were screened in 200 normolipidaemic controls using two independent High Resolution Melting (HRM) assays.

3. Results

Proband 1, a 39-year-old Pakistani male, was referred to the Lipid Unit of the University Hospital San Joan (Reus, Spain) when he presented with the fifth episode of acute pancreatitis. After that episode he developed insulin dependent diabetes. This patient had had the first episode of pancreatitis at the age of 23. The highest recorded TG level was 4489 mg/dL. During the last year, the patient has been treated with a low-fat diet supplemented with medium chain triglycerides (SHS, Nutricia) 30 mL/day, plus atorvastatin 10 mg, fenofibrate 145 mg and 3 g of omega-3 fatty acids. This treatment however, did not improve his clinical conditions nor did it reduce plasma TG levels below 1000 mg/dL. He did not show eruptive xanthomas, hepatosplenomegaly or lipemia retinalis. No family members were available for study although we could confirm that his parent were first cousins and he has four brothers (Figure 1).

Proband 2 is a 25-year-old female from Ecuador born from consanguineous parents (first cousins). She attended the hospital La Paz (Madrid, Spain) for follow up when she started treatment with low-fat diet supplemented with medium-chain triglycerides (Nutrición Médica, Madrid, Spain) 20 mL/day, plus fenofibrate 145 mg, 2 g of omega-3 fatty acids and 600 mg of crystalline niacin. No beneficial effects were obtained in clinical or biochemical terms. Since the age of 15 she had had 12 episodes of acute pancreatitis. The highest recorded TG level was 3820 mg/dL. The lowest TG level reported was 343 mg/dL when she managed to follow a strict vegetarian diet and do sport every day. Proband 2 is the eldest of four healthy siblings which were available for the study together with their mother (Figure 1). Eruptive xanthomas, hepatosplenomegaly or lipemia retinalis were not observed in this patient. There was no known family history of pancreatitis.
The biochemical and genetic data are shown in Table 1. At the time of the molecular diagnosis both probands had triglycerides levels above 1000 mg/dL. Both patients exhibited very low levels of post-heparin plasma LPL activity compared with hypertriglyceridaemic patients without LPL deficiency (92 mU/mL ± 44 mU/mL)\(^{22}\). Plasma ApoCII level was in the range found in our series of patients with severe hypertriglyceridemia\(^{23}\).

In both probands the sequence of the \(LPL\) as well as the sequence of the \(APOC2\) gene did not reveal the presence of rare variants nor polymorphisms that could account for the very low LPL activity (Supplementary table S1). The sequencing of \(GPIHBP1\) revealed two novel point mutations (Figure 2). Proband 1 was found to be homozygous for a C > A transversion in exon 2 resulting in the conversion of threonine to lysine at position 80 (p.Thr80Lys, p.T80K). Proband 2 was found to be homozygous for a G>T transversion in the third base of the ATG translation initiation codon in exon 1 resulting in the conversion of methionine to isoleucine (p.Met1Ile, p.M1I). According to \textit{in silico} analysis (PolyPhen-2) the Thr80Lys mutation was predicted to be possibly damaging and the Met1Ile probably damaging. According to SIFT algorithm both mutations were predicted to be damaging.

The sequencing of the first exon of \(GPIHBP1\) in the relatives of Proband 2 revealed that the mother (B.I-2. Figure 1) and the youngest brother (B.II-4) of the proband were heterozygous carriers of the mutation (Table 1). None of the two \(GPIHBP1\) mutations was found in the public data base from the National Center of Biotechnology, (http://www.ncbi.nlm.nih.gov); from the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php) nor from the 1000 Genomes Catalog of Human Genetic Variation (http://browser.1000genomes.org/index.html). In addition, no carriers of the mutations were identified in a group of normolipidaemic Spanish workers\(^{21}\). A summary of the mutations in \(GPIHBP1\) found in severe hypertriglyceridaemic patients so far is displayed in Table 2.

Additionally to the \(GPIHBP1\) mutation, Proband 2 was homozygous for the rare allele of the common SNP in \(APOA5\): rs3135506 (p.Ser19Trp), while her mother (B.I-2) was heterozygous and one of her brothers (B.II-2) was homozygous for this SNP (Table 1). Moreover, the youngest brother (B.II-4) was heterozygous not only for p.Ser19Trp variant but also for another SNP (rs662799; c.-1131C>T) in the \(APOA5\) gene. In all subjects the \(APOE\) genotype was ǫ3ǫ3.

4. Discussion

\(GPIHBP1\) has been demonstrated to be an essential factor for intravascular lipolysis of TG-rich lipoproteins mediated by LPL\(^{2}\). Recently different \(GPIHBP1\) missense mutations have been found in patients with Type I hypertriglyceridaemia. In the present study we describe two novel rare variants of \(GPIHBP1\) gene found in homozygous state in two patients with severe hypertriglyceridemia associated with extremely low LPL activity in post-heparin plasma.

The clinical features of our patients were similar to those of other adult patients with \(GPIHBP1\) mutations reported so far (Table 2). Our patients showed persistently elevated plasma TG that did not respond to lipid lowering treatment and had partial response to diet\(^{24,12-14,16,25-27}\). Most patients with severe hypertriglyceridemia carrying mutations in \(GPIHBP1\) suffer from pancreatitis bouts (62% of cases described in Table 2 with these data available), including very young children\(^{16,29,23}\) and, like in our probands, in some patients pancreatitis is reported to be recurrent\(^{13,16,17,24}\). On the other hand, we haven't observed in our patients other associated symptoms such as eruptive xantomas, lipemia retinalis or hepatosplenomegaly (45% of cases in Table 2). Finally, CHD is reported just in the two oldest patients described in Table 2\(^{13,24}\).
Proband 1 (Table 1) is homozygous for a non-conservative amino acid substitution (p.Thr80Lys) located in Finger 1 of the Ly6 domain of the GPIHBP1 protein. This is the first naturally occurring mutation in Finger 1 that does not affect a cysteine residue. The threonine residue at position 80 belongs to a N-glycosylation consensus sequence and is close to the cysteine residue at position 83. The substitution of an uncharged polar amino acid (threonine MW 119.13) with a positively charged polar amino acid that is larger in size (lysine MW146.19) is expected to disrupt the sequence of Finger 1 domain and to be deleterious. This prediction is supported by in vitro mutagenesis studies which demonstrated that p.Thr80Lys GPIHBP1 mutant, when expressed in CHOK1 cells, showed a reduced expression on the cell surface (suggesting an impaired intracellular transport) as well as a markedly reduced binding of LPL (> 90% reduction). In view of these findings we can reasonably conclude that p.Thr80Lys substitution is the cause of LPL deficiency in our patient.

The ATG (AUG) to ATT (AUU) conversion (leading to p.Met1Ile substitution) in the translation initiation codon found in Proband 2 is the first mutation affecting the GPIHBP1 translation initiation codon described so far. Mutations affecting the translation initiation codon in human genes are relatively uncommon as compared to other exonic mutations and usually are considered deleterious. Interestingly, this type of mutation has been reported in other genes involved in intravascular lipolysis of TG-rich lipoproteins such as LPL and APOC2.

The effect of the conversion of the ATG (AUG) initiation codon to ATT (AUU) may have a variable effect on the efficiency of translation. The mutation can result in: i) a complete block of translation initiation with no production of GPIHBP1; ii) a reduced efficiency of translation initiation by the mutant translation initiation codon (with a parallel reduction of the synthesis of Met1Ile mutant GPIHBP1); iii) an activation of one or more alternative translation initiation codons along the mRNA sequence with a variable translation efficiency. The visual inspection of GPIHBP1 mRNA sequence reveals the presence of ATG triplets at 106, 125, 130, 253 and 381 nucleotides downstream from the position +1 (corresponding to the Adenine of the canonical ATG). These ATG triplets might be regarded as possible translation initiation sites, if embodied in a nucleotide sequence similar to Kozak consensus initiation sequence [(GCC) GCC -3 A/GCC ATG +4 G]4. However, the activation of alternative translation initiation sites at position 106, 125 or 130 is out of frame with respect to the canonical ATG initiation site and would lead to the insertion of a premature termination codon (TGA at position 231).

Regardless of the possible effect of the ATG->ATT mutation on the translation process, Proband 2 showed a dramatic reduction of LPL activity suggesting either a very low production of the mutant GPIHBP1 carrying the Met1Ile mutation (due to a residual initiation translation efficiency) or the production of a structurally abnormal GPIHBP1 protein devoid of function.

As expected, plasma TG levels observed in the heterozygous relatives of proband 2 were in a normal range in agreement with many other cases described in the literature. A stringent follow up of these subjects is highly recommended as they (like heterozygotes for LPL or APOA5 gene mutations) may be prone to develop hypertriglyceridemia and be at risk of pancreatitis, when secondary factors such as diabetes, obesity, alcohol abuse or pregnancy should occur. In this context it is especially interesting the case of the youngest brother of proband 2 who has a normal TG level in spite of being not only a carrier of the GPIHBP1-Met1Ile mutation but also of two common APOA5 SNPs known to be associated with hypertriglyceridemia in adults as well as in children.

Our results highlight the importance of sequencing the GPIHBP1 gene in those patients with severe hypertriglyceridemia negative for mutations in LPL and APOC2. The number of GPIHBP1 mutations found in these patients has increased during the last few years suggesting that the GPIHBP1 gene mutations may be more frequent than previously assumed.
Authors’ contributions

MJA contributed to the study design, participated in the genetic analyses and drafted the manuscript. PLMH, DI, CGA and NP were in charge of the patient’s management and carried out the data collection. MJA and CR performed the sequencing of the GPIHBP1 gene and the in vitro analyses of the mutations. PT and SC participated in the design and coordination of the study and helped to draft the manuscript. JR carried out the plasma lipid analysis and the LPL activity assays. GO contributed to the LPL activity assays and helped to draft the manuscript. PV conceived the study, participated in its coordination and helped to draft the manuscript. All authors read and approved the final version of the manuscript.

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References


Title and legend to figures

Figure 1. Pedigrees of proband 1 (A) and proband 2 (B). Probands are designated with filled symbols. The question marks (?) denote individuals who could not be studied.

Figure 2. GPIHBP1 sequences showing the mutations found in our patients. Panel 1.a shows the normal sequence and 1.b the homozygous C>A transversion at the second base of codon 80 in exon 3, resulting in a conversion of threonine to lysine found in proband 1. Panel 2.a shows the normal sequence and 2.b the homozygous G>T transversion at the third base of codon 1 in exon 1, resulting in a conversion of the first methionine to isoleucine found in proband 2. The sequence in panel 2.c corresponds to the youngest brother of proband 2 who is heterozygous for the mutation.
Table 1. Anthropometric, biochemical and genetic data

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<thead>
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<th>Subject</th>
<th>Age</th>
<th>BMI  (Kg/m2)</th>
<th>TC  (mg/dL)</th>
<th>HDL-C (mg/dL)</th>
<th>TG  (mg/dL)</th>
<th>Max. TG (mg/dL)</th>
<th>ApoCII (mg/dL)</th>
<th>LPL activity (mU/mL)</th>
<th>Mutation in GPIHBP1</th>
<th>SNP(s)</th>
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<td>15</td>
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<td>4489</td>
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<td>9.5</td>
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**Subject:** P: proband. The subject’s code according to the pedigrees displayed in figure 1 is indicated in parentheses. **BMI:** body mass index. **TC:** total cholesterol. **TG:** triglycerides. **HDL-C:** cholesterol in high density lipoproteins. **Max. TG:** highest level of triglyceride reported. **ApoCII:** Apolipoprotein C2. **SNPs:** Single nucleotide polymorphisms. APOE (rs429358 and rs7412, alleles ε2, ε3 and ε4) and APOA5 (rs662799 and rs3135506) common variants were screened.
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<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>&gt;886</td>
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<td>Report</td>
<td>Mutation (HO: homozygous; CHE: compound heterozygous)</td>
<td>Patient code</td>
<td>Gender, age</td>
<td>Origin</td>
<td>PC</td>
<td>Pancreatitis (age of onset)</td>
<td>Associated symptoms</td>
<td>Max. TG</td>
<td>Min. TG</td>
</tr>
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<td>Berge K.E. et al.; 2014</td>
<td>c.182-_555+?del (Ex3-4 del), p.0 (HO)</td>
<td>VI:2</td>
<td>F, 22 y</td>
<td>Pakistani</td>
<td>Yes</td>
<td>RP</td>
<td>NA</td>
<td>5314</td>
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<td>c.182-_555+?del (Ex3-4 del), p.0 (HO)</td>
<td>VI:1</td>
<td>M, 37 y</td>
<td>Pakistan</td>
<td>Yes</td>
<td>RP</td>
<td>NA</td>
<td>8857</td>
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<td>c.182-_555+?del (Ex3-4 del), p.0 (HO)</td>
<td>VI:3</td>
<td>M, 40 y</td>
<td>Pakistani</td>
<td>Yes</td>
<td>No</td>
<td>NA</td>
<td>&gt;3543</td>
<td>NA</td>
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<td>VI:4</td>
<td>F, 37 y</td>
<td>Pakistan</td>
<td>Yes</td>
<td>RP</td>
<td>NA</td>
<td>2391</td>
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<td>Plengpanich W. et al., 2014</td>
<td>c.320C&gt;G, p.S107C (HO)</td>
<td>II-8</td>
<td>F, 46 y</td>
<td>Thai</td>
<td>No</td>
<td>Abdominal pain</td>
<td>NO</td>
<td>6448</td>
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<td>c.320C&gt;G, p.S107C (HO)</td>
<td>II-1</td>
<td>M, 64 y</td>
<td>Thai</td>
<td>No</td>
<td>NA</td>
<td>NA</td>
<td>842</td>
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<td>c.320C&gt;G, p.S107C (HO)</td>
<td>II-10</td>
<td>M, 43 y</td>
<td>Thai</td>
<td>No</td>
<td>NA</td>
<td>NA</td>
<td>673</td>
<td>NA</td>
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<tr>
<td>Ahmad Z. et al., 2014</td>
<td>c.267C&gt;A, p.C89* + c.85-88GAGGdel, pE29Tfs*50 (CHE)</td>
<td>P</td>
<td>F, 6 m</td>
<td>Caucasian</td>
<td>No</td>
<td>Yes</td>
<td>EX</td>
<td>2665</td>
<td>423</td>
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<tr>
<td>This report</td>
<td>c.239 C&gt;A, p.T80K (HO)</td>
<td>A.II-1</td>
<td>M, 37 y</td>
<td>Pakistani</td>
<td>Yes</td>
<td>RP (23 y)</td>
<td>NO</td>
<td>4489</td>
<td>1000</td>
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<td>c.3 G&gt;T, p.M11 (HO)</td>
<td>B.II-1</td>
<td>F, 25 y</td>
<td>Ecuatorian</td>
<td>Yes</td>
<td>RP (15 y)</td>
<td>NO</td>
<td>3820</td>
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NA (throughout the table): data not available. **Patient code:** code given to each particular patient in the original reference. When there are more than one member of the same family the proband is listed the first. **Gender and age:** M: male, F: female, y: years, m: months, w: weeks, d: days. **PC:** reported parent’s consanguinity. In references 24 and 27 common ancestors are suspected. **Pancreatitis:** it is indicated whether the patient suffered or not from pancreatitis (Yes/ No) or just abdominal pain. **RP:** recurrent pancreatitis. The age of the first episode is indicated in parentheses when available. **Associated symptoms:** CHD: coronary heart disease, NO: not observed, HSM: hepatosplenomegaly, LR: lipemia retinalis, SM: splenomegaly. **Max. TG:** highest triglyceride level reported for each patient. **Min. TG:** lowest triglyceride level achieved for each patient under different treatments and formula diets. The common GPIHBP1 variant p.C14F, rs11538389 is described in references 13 and 16 and the variant p.S144F, rs78367243 is found in reference 14. References 13, 16, 17, 23-28 and this report include information on LPL activity. ApoCII levels are given in references 13, 23, 25, 26 and in this report. **In vitro** functional analyses of the mutations are carried out in reports 12, 13, 16, 25-27.
1.a

G/G

2.a

C/C

2.b

T/T

2.c

A/A

T/G


We studied two patients with recurrent pancreatitis and severe hypertriglyceridemia. Patients exhibited low lipoprotein lipase activity but no rare variants in this gene. We identified two novel missense mutations in the \textit{GPIHBP1} gene. One patient was homozygous for the mutation c.239 C>A, ACG>AAG, p.Thr_{80}>Lys. The other patient was homozygous for the mutation c.3 G>T, ATG>ATT, p.Met_{1}>Ile.
**Supplementary Table S1**

### LPL sequencing

<table>
<thead>
<tr>
<th>Proband</th>
<th>Variants Region State</th>
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<tr>
<td>Proband 1</td>
<td>No variants found - -</td>
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<tr>
<td>Proband 2</td>
<td>c.1164 C&gt;A, ACC&gt;ACA, Thr361&gt;Thr Exon 8 Heterozigosity</td>
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</table>

### APC2 sequencing

<table>
<thead>
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<th>Proband</th>
<th>Variants Region State</th>
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<tr>
<td>Proband 1</td>
<td>IVS3+38_40 del 3bp (ACC) IVS3 Homozygosity</td>
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<tr>
<td>Proband 2</td>
<td>UTR5’-109 G&gt;C UTR 5’ Heterozigosity</td>
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<td>UTR 5’ -24 G&gt;T UTR 5’ Heterozigosity</td>
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<tr>
<td></td>
<td>IVS1-67 G&gt;T IVS1 Homozygosity</td>
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<tr>
<td></td>
<td>IVS3+38_40 del 3bp IVS3 Heterozigosity</td>
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<tr>
<td></td>
<td>IVS3-151 C&gt;G IVS3 Homozygosity</td>
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<td>IVS3-150_-148 del 3bp IVS3 Heterozigosity</td>
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<td>IVS3-81 C&gt;T IVS3 Heterozigosity</td>
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