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Characterization of two novel pathogenic variants at compound heterozygous status in Lipase Maturation Factor 1 (*LMF1*) gene causing severe hypertriglyceridemia

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Abstract

Background: Severe hypertriglyceridemia is a rare disease characterized by triglyceride levels higher than 1000 mg/dl (11.3 mmol/L) and acute pancreatitis. The disease is caused by pathogenic variants in genes encoding lipoprotein lipase (*LPL*), apolipoprotein A5 (*APOA5*), apolipoprotein C2 (*APOC2*), glycosylphosphatidylinositol-anchored high density lipoprotein binding protein 1 (*GPIHBP1*) and lipase maturation factor 1 (*LMF1*).

Objective: We aim to identify the genetic cause of severe hypertriglyceridemia and characterize the new variants in a patient with severe hypertriglyceridemia.

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Declaration of interest: none

Contribution Statement: Miklós Péterfy coordinated the functional experiments, analyzed results and co-write the paper; Candy Bedoya performed functional experiments; Carola Giacobbe performed genetic analysis and interpreted data; Carmen Pagano examined and followed patients; Marco Gentile collected patient samples and made biochemical evaluation; Paolo Rubba examined and followed patients; Giuliana Fortunato coordinated genetic screening, analyzed results and co-write the paper; Maria Donata Di Taranto performed genetic analysis, analyzed results and co-write the paper. All authors approved the final version of the manuscript.

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Methods: The proband was a male showing severe hypertriglyceridemia (triglycerides 1416 mg/dL, 16.0 mmol/L); proband's relatives were also screened. Genetic screening included direct sequencing of the above genes and identification of large rearrangements in the *LPL* gene. Functional characterization of mutant LMF1 variants was performed by complementing LPL maturation in transfected Lmf1-deficient mouse fibroblasts.

Results: The proband and his affected brother were compound heterozygotes for variants in the *LMF1* gene never identified as causative of severe hypertriglyceridemia c.[157delC;1351C>T]; [410C>T], p.[(Arg53Glyfs*5)];[(Ser137Leu)]. Functional analysis demonstrated that the p.(Arg53Glyfs*5) truncation completely abolished and the p.(Ser137Leu) missense variant dramatically diminished the lipase maturation activity of LMF1.

Conclusions: In addition to a novel truncating variant, we describe for the first time a missense variant functionally demonstrated affecting the lipase maturation function of LMF1. This is the first case in which compound heterozygous variants in *LMF1* was functionally demonstrated as causative of severe hypertriglyceridemia.

Keywords

severe hypertriglyceridemia; LPL activity; genetic screening; functional assay; pathogenicity evaluation; Lipase Maturation Factor-1 (LMF1)

INTRODUCTION

Severe hypertriglyceridemia is a rare disease characterized by triglyceride levels higher than 1000 mg/dl (11.3 mmol/L). The main clinical consequences of high triglycerides are acute pancreatitis, abdominal pain, cutaneous xanthomas, hepatosplenomegaly and lipaemia retinalis^{1, 2}. The principal molecular defect underlying hypertriglyceridemia is defective hydrolysis of triglycerides contained in triglyceride-rich lipoproteins, such as chylomicrons and very low density lipoproteins (VLDL). Lipoprotein lipase (LPL) is the key enzyme of triglyceride hydrolysis and mutations in the *LPL* gene as well as in genes encoding other proteins needed for LPL function are recognized as genetic causes of severe hypertriglyceridemia^{2, 3}. Mutations in *LPL* or the gene encoding apolipoprotein A5 (*APOA5*) are the most frequent causes, whereas a few cases have been ascribed to mutations in genes encoding apolipoprotein C2 (*APOC2*), glycosylphosphatidylinositol-anchored high density lipoprotein-binding protein 1 (*GPIHBP1*), and lipase maturation factor-1 (*LMF1*). *APOA5* and *APOC2* are contained in triglyceride-rich lipoproteins and modulate the activity of LPL³. *LMF1* is required for LPL folding and/or dimerization, while *GPIHBP1* is an anchor protein that retains LPL at the endothelial surface⁴⁻⁶. Only few families with severe hypertriglyceridemia due to *LMF1* causative variants have been described so far⁷⁻⁹.

Recent guidelines suggest to carefully evaluate evidence about genetic variants in order to claim their pathogenicity¹⁰. The experimental demonstration of functional defect of the protein carrying the variant is considered as "strong evidence" of pathogenicity, whereas bioinformatics predictions should be considered only as supporting criteria^{10, 11}. To date, only nonsense variants of *LMF1* have been clearly identified in functional assays as disease causing, whereas no functional defects have so far been associated with missense *LMF1*

variants^{4, 12}. Here, we aim to characterize two novel variants found at compound heterozygous status in a patient suffering from severe hypertriglyceridemia.

MATERIALS AND METHODS

Patient

The proband was a male reporting the first documented severe hypertriglyceridemia at the age of 36 years (about 2000 mg/dL - 22.58 mmol/L). No additional triglycerides measurements were performed until the age of 44 years, when he encountered abdominal pain and was referred to the Department of Clinical Medicine and Surgery of the University of Naples Federico II. Proband relatives were also examined. The study was performed according to the current version of the Helsinki Declaration and was approved by the Ethical Committee of the Università degli Studi di Napoli Federico II (Number 157/13, September 9, 2013). Informed consent was obtained from the patient and his relatives.

Clinical and biochemical analysis

Physical examination was performed by evaluating the body mass index (BMI), presence of eruptive xanthomas, presence of abdominal pain and the general condition of the patient. Biochemical measurements were carried out on serum collected after an overnight fast. The automated analyzer Modular P3 analyzer (Roche Diagnostics, USA) was used to measure levels of total plasma cholesterol and triglycerides by standard methods and HDL-cholesterol by a homogeneous enzymatic colorimetric assay. When possible, the Friedewald formula was used to calculate LDL-cholesterol levels.

LPL mass was measured by enzyme-linked immunosorbent assay using colorimetric detection at the Unilabs laboratory (Unilabs, Denmark). Lipase activity in post-heparin plasma and cell culture media was measured using a fluorometric lipase activity assay kit (Cell Biolabs, USA). The assay detects the activities of all three LMF1-dependent lipases including LPL, hepatic lipase and endothelial lipase.

Genetic screening

Genetic screening was performed by PCR amplification and direct sequencing of the promoter and all exons with flanking intronic regions of *LPL* (NM_000237), *APOA5* (NM_052968), *APOC2* (NM_000483), *GPIHBP1* (NM_178172) and *LMF1* (NM_022773) genes. Reaction mixtures and sequence alignment by CodonCode Aligner (CodonCode corporation, USA) have been assembled as previously described¹³. Large rearrangements in the *LPL* gene were investigated by Multiplex Ligation-dependent Probe Amplification (MLPA - MRC-Holland, The Netherlands), as previously reported¹⁴. Variant nomenclature follows the Human Genome Variation Society (HGVS) recommendations (<http://varnomen.hgvs.org/>). Variants were checked against the Human Gene Mutation Database (HGMD) and the variant database dbSNP 149 (NCBI) together with the following next generation databases to evaluate Minor Allele Frequency (MAF): Exome Aggregation Consortium (ExAC), genome Aggregation Database (gnomAD), Exome Variant Server (EVS) and 1000 genomes (1kG). It is important to note that all ExAC data are included in the gnomAD database.

Pathogenicity evaluations were performed following the American College of Medical Genetics and Genomics (ACMG) guidelines ¹⁰.

Bioinformatics predictions for the missense variant included PolyPhen-2 (Polymorphism Phenotyping v2), MutationTaster, Sorting Intolerant from Tolerant (SIFT), Mutation Assessor and Protein Variation Effect Analyzer (PROVEAN) ¹¹.

Immunoblot analysis of LMF1

The impact of mutations on LMF1 protein expression was evaluated by immunoblot analysis in HEK293 cells. Mutations were generated by site-directed mutagenesis using myc- epitope-tagged LMF1 constructs. One day after cotransfection with mixtures of mutant LMF1 plasmids and a Gaussia luciferase expression plasmid for normalization, cell lysates were assayed for luciferase activity using the GARI substrate (Targeting Systems, USA). Cell lysates representing equal luciferase counts were separated by SDS-PAGE and immunoblotted with HRP-conjugated anti-myc antibody (9E10, Thermo Fisher, USA) at 1:250,000 dilution.

Functional analysis of LMF1 variants

LMF1 variants were functionally analyzed using a second-generation LMF1 activity assay based on the reconstitution of LPL maturation in LMF1-deficient HEK293 cells generated by CRISPR-mediated mutagenesis ¹⁵. Cells were cotransfected in quintuplicates with mixtures of V5-LPL, wild-type or mutant *LMF1* constructs generated by site-directed mutagenesis and a firefly luciferase vector (pGL3-Control, Promega, USA) for transfection normalization. To allow precise quantitative assessment of LMF1 protein levels, LMF1 variants were expressed as fusion proteins with Gaussia luciferase at the N-terminus (Gluc-LMF1). At 24 h posttransfection, 10 U/ml heparin was added to media and after 30 min cells were incubated with a fresh aliquot of heparin-containing media for 2 hours. Media were harvested for fluorometric LPL activity assay (Cell Biolabs, USA) and immunoblot analysis, and cells were lysed for dual-luciferase reporter assay (Promega, USA). LMF1 activity was calculated as the ratio of LPL activity in the media and intracellular LMF1 protein amount (i.e. Gaussia luciferase activity). For the analysis of LPL mass in cell culture media, 5 µl samples were separated by SDS-PAGE and immunoblotted with anti-V5 (1:10,000; Life Technologies, USA) and HRP-conjugated rabbit anti-mouse IgG (1:100,000; Jackson ImmunoResearch, USA) antibodies.

RESULTS

Clinical evaluation

At his first visit at our lipid clinic, the proband was overweight (BMI of 28.5 Kg/m²) and showed 1416 mg/dL (16.0 mmol/L) triglycerides, 182 mg/dL (4.7 mmol/L) total cholesterol and 22 mg/dL (0.6 mmol/L) HDL-cholesterol. Thyroid parameters were in the normal range and diabetes mellitus, alcoholism, renal disease and autoimmune diseases were excluded. Abdominal ultrasound examination revealed mild hepatic steatosis (bright liver) with hepatosplenomegaly, whereas no signs of eruptive xanthomas were found. The familial anamnesis revealed that one brother out of 5 siblings suffered from severe

hypertriglyceridemia (>1200 mg/dL - >13.5 mmol/L) and had one attack of acute pancreatitis. The proband's affected brother was obese and exhibited the following lipid profile in therapy with 2 g/day omega-3 fatty acids, 40 mg/day atorvastatin and 600 mg/day ursodeoxycholic acid for gallstones: 521 mg/dL (5.9 mmol/L) triglycerides, 101 mg/dL (2.6 mmol/L) total cholesterol, 13 mg/dL (0.3 mmol/L) HDL-cholesterol and 75 mg/dL (1.9 mmol/L) LDL-cholesterol.

None of the other siblings showed hypertriglyceridemia, whereas mild hypertriglyceridemia was observed in the patient's parents, in particular in the mother who was overweight and also suffered from diabetes and subclinical hypothyroidism.

After 3 months of discontinuous therapy with 3 g/day omega-3 fatty acids and 145 mg/day fenofibrate combined with a hypolipidemic/hypocaloric diet, the proband still showed high triglycerides [898 mg/dL (10.1 mmol/L)], 157 mg/dL (4.1 mmol/L) total cholesterol and 19 mg/dL (0.5 mmol/L) HDL-cholesterol. LPL mass measured in the patient's plasma after heparin infusion was 229 ng/ml, which corresponds to 81.7% of normal levels, whereas total lipase activity was 14.9 mU/mL, which corresponds to 29.1% of normal levels. At the time of LPL mass evaluation the patient discontinued therapy and diet on his own decision resulting in moderately high triglycerides (491 mg/dL; 5.5 mmol/L), normal total cholesterol (178 mg/dL, 4.6 mmol/L) and low HDL-cholesterol (27 mg/dL, 0.7 mmol/L).

After a year of better compliance with therapy and diet, he exhibited normal triglyceride levels [172 mg/dL (1.9 mmol/L)], 142 mg/dL (3.7 mmol/L) total cholesterol, 23 mg/dL (0.6 mmol/L) HDL-cholesterol and 85 mg/dL (2.2 mmol/L) LDL-cholesterol.

Genetic screening

Based on the clinical presentation and suspicion of type 1 hyperlipoproteinemia, we performed genetic screening of the *LPL*, *APO5*, *APOC2*, *GPIHBP1* and *LMF1* genes in the proband. In addition to common variants in the first four genes, 3 rare variants were identified in *LMF1*. Subsequent extension of the analysis to the patient's relatives revealed the allelic relationships among these variants. The proband (II.2 in Figure 1) is compound heterozygote carrying rare variants on both alleles of *LMF1*: c.[157delC;1351C>T]; [410C>T], p.[(Arg53Glyfs*5)];[(Ser137Leu)], where the variants included in the squared bracket are present on the same allele (Figure 1). Deletion of a single base at c.157delC produces frameshift with a premature stop codon in the protein, p.(Arg53Glyfs*5). p.(Arg53Glyfs*5) is a new variant absent from HGMD or variant databases containing data from next generation sequencing. Furthermore, p.(Arg53Glyfs*5) is expected to be a null-mutation, as the premature stop codon results in a C-terminally truncated protein lacking both the lipase interaction and maturation domains (Figure 2A), regions that are critical for LMF1 function¹⁶. Due to the frameshift and early termination of the protein, the c.157delC deletion makes ineffective the other variant on the same allele (c.1351C>T) that would have resulted in the missense variant p.(Arg451Trp).

The other allele carried the single base substitution c.410C>T leading to the very rare missense variant p.(Ser137Leu), which has not been previously associated with hypertriglyceridemia. p.(Ser137Leu) (rs549008037) is present at very low frequencies in

variant databases: 3 heterozygotes in ExAC (MAF=0.00003); 5 heterozygotes in gnomAD (MAF=0.00002); 1 heterozygote in 1kG (MAF=0.0008). Ser137 is localized within the second transmembrane domain of LMF1 (Figure 2A) and its perfect evolutionary conservation from humans to invertebrates (Figure 2B) suggests that any substitution at this position would result in impaired LMF1 function.

The proband's hypertriglyceridemic brother was also identified as compound heterozygote (II.1 subject in Figure 1), whereas his unaffected siblings, parents and daughters are heterozygotes. The co-segregation of compound heterozygosis and phenotype suggests that only the simultaneous presence of *LMF1* variants affecting both alleles may be causative of severe hypertriglyceridemia.

All bioinformatics predictions suggested a pathogenic role for p.(Ser137Leu): 'Probably damaging' in PolyPhen-2 (both HumDiv and HumVar), 'Disease causing' in MutationTaster, 'Damaging' in SIFT, 'High functional impact' in Mutation Assessor, and 'Deleterious' in PROVEAN.

Based on ACMG criteria for pathogenicity assessment ¹⁰, we classified the c.[157delC; 1351C>T], p.(Arg53Glyfs*5) variant as "pathogenic" (criteria PVS1, PM2, PM4, PP1), whereas the status of variant c.410C>T, p.(Ser137Leu) remains "of uncertain significance" (criteria PM2, PP1 and PP3).

Functional characterization of LMF1 variants

Immunoblot analysis demonstrated that the p.(Ser137Leu) and p.(Arg53Glyfs*5) mutations dramatically diminish the expression of LMF1 (Figure 2C). In contrast, a previously characterized truncation p.(Trp464Ter) ⁸ limited to the C-terminal domain of LMF1 was expressed at levels similar to the wild-type protein (Figure 2C). To investigate the effects of p.(Arg53Glyfs*5) and p.(Ser137Leu) variants on LMF1 function, we assessed their impact in a quantitative assay of LPL maturation ¹⁵. Whereas the p.(Arg53Glyfs*5) mutant was completely inactive, the p.(Ser137Leu) variant exhibited dramatically reduced, but detectable LPL activity and LMF1 specific activity similar to the truncated p.(Trp464Ter) protein characterized previously (Figure 2D and 2E) ⁸. Immunoblot analysis of the cell culture media revealed marked reduction and complete deficiency in LPL secretion by cells expressing p.(Ser137Leu) and p.(Arg53Glyfs*5), respectively (Figure 2F). This result is consistent with the observation of reduced post-heparin plasma LPL mass in the proband. In conclusion, the functional assays demonstrated that both p.(Arg53Glyfs*5) and p.(Ser137Leu) result in major impairment of the lipase-maturation activity of LMF1 and that the presence of both variants at compound heterozygous status leads to reduced active LPL, explaining the severe hypertriglyceridemia in the proband. Furthermore, based on the additional criterion of functional validation (PS3), p.(Ser137Leu) is now considered a "likely pathogenic" variant according to ACMG classification ¹⁰.

DISCUSSION

Since the identification of LMF1 as a chaperone required for the post-translational maturation of LPL, the *LMF1* gene has emerged as a genetic determinant of

hypertriglyceridemia in diverse populations. Although, an accumulation of heterozygous *LMFI* rare variants has been previously observed in patients with hypertriglyceridemia, these variants are also often present in healthy subjects and their pathogenicity remains untested^{12, 17, 18}. Thus far, only 4 hypertriglyceridemic patients have been described as carriers of rare variants on both alleles of the *LMFI* gene: 3 homozygotes for nonsense variants^{7–9} and a compound heterozygote harboring a nonsense and a missense variant¹⁹, although the latter has not been functionally characterized. The three homozygous *LMFI* truncating mutations [p.(Tyr430Ter), p.(Tyr460Ter), p.(Trp464Ter)] have previously been experimentally demonstrated to impact on LMF1 activity^{7–9}. Until now, functional studies confirmed the pathogenic role of only nonsense variants in *LMFI*, whereas none of the missense variants analyzed thus far affected the function of this protein^{7, 8, 12}. In the present study, we report the identification and functional characterization of two rare *LMFI* variants - p.(Arg53Glyfs*5) and p.(Ser137Leu) - that have not been linked to dyslipidemia before and occur at compound heterozygosity in an individual with severe hypertriglyceridemia. Using a quantitative assay of LMF1 activity, we demonstrated that both variants profoundly impair the secretion of active LPL enzyme. The truncated p.(Arg53Glyfs*5) LMF1 protein had no detectable lipase maturation activity indicating that it is a null allele. Furthermore, our functional studies identified p.(Ser137Leu) as the first missense mutation affecting LMF1 function reported to date. This variant severely diminished the expression of mutant LMF1 most likely by reducing the stability and promoting the turnover of the protein. In addition, the p.(Ser137Leu) mutation dramatically reduced the specific activity of LMF1. Our *in vitro* results suggest that compound heterozygous p.[(Arg53Glyfs*5)];[(Ser137Leu)] cells produce less than 5% LPL activity relative to wild-type cells. Consistent with this conclusion, post-heparin plasma lipase activity in the proband was 29.1% of healthy controls. The more modest lipase deficiency observed *in vivo* likely reflects the presence of hepatic lipase, which is significantly less affected by LMF1 deficiency than LPL^{7, 8, 12}. Based on these results, we conclude that compound heterozygosity for p.[(Arg53Glyfs*5)];[(Ser137Leu)] is the most likely cause of hypertriglyceridemia in the proband.

These data highlight that functional characterization is required to claim pathogenicity of variants associated with severe hypertriglyceridemia as well as other forms of genetic dyslipidemias²⁰. Therefore the functional characterization should integrate genetic screening especially in case of variants of uncertain significance.

CONCLUSIONS

We demonstrated for the first time that two novel compound heterozygous mutations in *LMFI* are responsible for severe hypertriglyceridemia. p.(Ser137Leu) represents the only functional missense variant identified in LMF1 to date. Our results will contribute to improved genetic diagnosis of severe hypertriglyceridemia.

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References

1. Brahm A, Hegele RA. Hypertriglyceridemia. *Nutrients*. 2013;5:981–1001. [PubMed: 23525082]
2. Brahm AJ, Hegele RA. Chylomicronaemia--current diagnosis and future therapies. *Nature reviews. Endocrinology* 2015;11:352–362.
3. Ramasamy I Update on the molecular biology of dyslipidemias. *Clinica chimica acta; international journal of clinical chemistry*. 2016;454:143–185. [PubMed: 26546829]
4. Peterfy M Lipase maturation factor 1: a lipase chaperone involved in lipid metabolism. *Biochimica et biophysica acta*. 2012;1821:790–794. [PubMed: 22063272]
5. Fong LG, Young SG, Beigneux AP, et al. GPIHBP1 and Plasma Triglyceride Metabolism. *Trends Endocrin Met*. 2016;27:455–469.
6. Ehrhardt N, Bedoya C, Peterfy M. Embryonic viability, lipase deficiency, hypertriglyceridemia and neonatal lethality in a novel LMF1-deficient mouse model. *Nutrition & metabolism*. 2014; 11:37. [PubMed: 25302068]
7. Peterfy M, Ben-Zeev O, Mao HZ, et al. Mutations in LMF1 cause combined lipase deficiency and severe hypertriglyceridemia. *Nature genetics*. 2007;39:1483–1487. [PubMed: 17994020]
8. Cefalu AB, Noto D, Arpi ML, et al. Novel LMF1 nonsense mutation in a patient with severe hypertriglyceridemia. *The Journal of clinical endocrinology and metabolism*. 2009;94:4584–4590. [PubMed: 19820022]
9. Cefalu AB, Spina R, Noto D, et al. Identification of a novel LMF1 nonsense mutation responsible for severe hypertriglyceridemia by targeted next-generation sequencing. *Journal of clinical lipidology*. 2017;11:272–281 e278. [PubMed: 28391895]
10. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genetics in medicine : official journal of the American College of Medical Genetics*. 2015;17:405–424. [PubMed: 25741868]
11. Camastra F, Di Taranto MD, Staiano A. Statistical and Computational Methods for Genetic Diseases: An Overview. *Computational and mathematical methods in medicine*. 2015;2015:954598. [PubMed: 26106440]
12. Surendran RP, Visser ME, Heemelaar S, et al. Mutations in LPL, APOC2, APOA5, GPIHBP1 and LMF1 in patients with severe hypertriglyceridaemia. *Journal of internal medicine*. 2012;272:185–196. [PubMed: 22239554]
13. Romano M, Di Taranto MD, Mirabelli P, et al. An improved method on stimulated T-lymphocytes to functionally characterize novel and known LDLR mutations. *Journal of lipid research*. 2011;52:2095–2100. [PubMed: 21865347]
14. Ruotolo A, Di Taranto MD, D'Agostino MN, et al. The novel variant p.Ser465Leu in the PCSK9 gene does not account for the decreased LDLR activity in members of a FH family. *Clinical chemistry and laboratory medicine*. 2014;52:e175–178. [PubMed: 24607922]
15. Yin F, Doolittle MH, Peterfy M. A quantitative assay measuring the function of lipase maturation factor 1. *Journal of lipid research*. 2009;50:2265–2269. [PubMed: 19471043]
16. Doolittle MH, Ehrhardt N, Peterfy M. Lipase maturation factor 1: structure and role in lipase folding and assembly. *Current opinion in lipidology*. 2010;21:198–203. [PubMed: 20224398]
17. Johansen CT, Wang J, McIntyre AD, et al. Excess of rare variants in non-genome- wide association study candidate genes in patients with hypertriglyceridemia. *Circulation. Cardiovascular genetics*. 2012;5:66–72. [PubMed: 22135386]
18. Lamiquiz-Moneo I, Blanco-Torrecilla C, Bea AM, et al. Frequency of rare mutations and common genetic variations in severe hypertriglyceridemia in the general population of Spain. *Lipids in health and disease*. 2016;15:82. [PubMed: 27108409]
19. De Castro-Oros I, Civeira F, Pueyo MJ, et al. Rare genetic variants with large effect on triglycerides in subjects with a clinical diagnosis of familial vs nonfamilial hypertriglyceridemia. *Journal of clinical lipidology*. 2016;10:790–797. [PubMed: 27578109]

20. Di Taranto MD, D'Agostino MN, Fortunato G. Functional characterization of mutant genes associated with autosomal dominant familial hypercholesterolemia: integration and evolution of genetic diagnosis. *Nutrition, metabolism, and cardiovascular diseases : NMCD*. 2015;25:979–987.

Highlights

- We described the first *LMFI* missense variant affecting LPL maturation
- *LMFI* variants at compound heterozygous status cause severe hypertriglyceridemia
- Functional characterization of new variants integrated genetic diagnosis

LMF1 c.410C>T - p.(Ser137Leu)

LMF1 c.[157delC;1351C>T] - p.(Arg53Glyfs*5)

Figure 1. Pedigree of the family with two cases of severe hypertriglyceridemia caused by *LMF1* mutations.

The proband is indicated by arrow. Subjects II.3 and II.5 have not been analyzed. Lipid values are expressed as mg/dL and mmol/L; age is shown in years. Lipid values were obtained in the absence of therapy for all subjects with the exception of subject II.1, who was in therapy with 40mg/day atorvastatin, 2g/day omega-3 fatty acids and 600 mg/day ursodeoxycholic acid.

Figure 2. Functional analysis of LMF1 mutations.

(A) Schematic illustration of LMF1 membrane topology and the location of p.Arg53Glyfs*5 and p.Ser137Leu mutations (red circles). Heavy line represents the DUF1222 domain and functional domains involved in lipase interaction and maturation are indicated by arrows. TM, transmembrane domain. (B) Evolutionary conservation of TM2. Amino acids identical to the human sequence (top) are represented by dots. Fully conserved residues across 42 orthologous LMF1 sequences, only a representative subset of which is shown, appear in bold. Ser137 is highlighted in red. H.s., Homo sapiens (human); M.m., Mus musculus

(house mouse); B.t., *Bos taurus* (cattle); E.c., *Equus caballus* (horse); G.g., *Gallus gallus* (chicken); A.m., *Apteryx mantelli* (kiwi); D.r., *Danio rerio* (zebrafish); P.n., *Pygocentrus nattereri* (red-bellied piranha); C.m., *Callorhynchus milii* (elephant shark); N.s., *Neomonachus schauinslandi* (Hawaiian monk seal); A.c., *Anolis carolinensis* (green anole); B.f., *Branchiostoma floridae* (Florida lancelet); S.p., *Strongylocentrotus purpuratus* (sea urchin). (C) LMF1 protein expression in cell lysates of cells transfected with wild-type (WT) and mutant *LMF1* constructs. (D) Activity of LPL secreted into the culture medium of transfected LMF1-deficient cells. (E) Specific activity of WT and mutant LMF1 proteins. (F) LPL protein secreted into the culture media by LMF1- deficient cells transfected with WT and mutant constructs. ‘no tfxn’, no LMF1 transfected *, $p < 0.05$; N.D., not detectable.