The official journal of the Japan Atherosclerosis Society and
the Asian Pacific Society of Atherosclerosis and Vascular Diseases

Original Article

J Atheroscler Thromb, 2019; 26: 136-144. http://doi.org/10.5551/jat.44537

A Compound Heterozygous Mutation of Lipase Maturation Factor 1 is Responsible for Hypertriglyceridemia of a Patient

Yihui Liu[1], Jiang Xu[2], Wanyun Tao[3], Rong Yu[4] and Xinjiang Zhang[1, 2]

[1]Department of Neurology, Affiliated Hospital of Yangzhou University, Yangzhou, China
[2]Medical School of Yangzhou University, Yangzhou, China
[3]Department of Biochemistry, School of Medicine, Case Western Reserve University Cleveland, USA.
[4]Department of Anesthesiology, the Second XiangYa Hospital, Central South University, Changsha, China

Aim: Dyslipidemia is the most common lipid metabolism disorder in humans, and its etiology remains elusive. Hypertriglyceridemia (HTG) is a type of dyslipidemia that contributes to atherosclerosis and coronary heart disease. Previous studies have demonstrated that mutations in lipoprotein lipase (*LPL*), apolipoprotein CII (*APOC2*), apolipoprotein AV (*APOA5*), glycosylphosphatidylinositol anchored high-density lipoprotein-binding protein 1 (*GPIHBP1*), lipase maturation factor 1 (*LMF1*), and glycerol-3 phosphate dehydrogenase 1 (*GPD1*) are responsible for HTG by using genomic microarrays and next-generation sequencing. The aim of this study was to identify genetic lesions in patients with HTG.

Method: Our study included a family of seven members from Jiangsu province across three generations. The proband was diagnosed with severe HTG, with a plasma triglyceride level of 38.70 mmol/L. Polymerase chain reaction (PCR) and Sanger sequencing were performed to explore the possible causative gene mutations for this patient. Furthermore, we measured the post-heparin LPL and hepatic lipase (HL) activities using an antiserum inhibition method.

Results: A compound heterozygous mutation in the *LMF1* gene (c.257C>T/p.P86L and c.1184C>T/p.T395I) was identified and co-segregated with the affected patient in this family. Both mutations were predicted to be deleterious by three bioinformatics programs (Polymorphism Phenotyping-2, Sorting Intolerant From Tolerant, and MutationTaster). The levels of the plasma post-heparin LPL and HL activities in the proband (57 and 177 mU/mL) were reduced to 24% and 75%, respectively, compared with those assayed in the control subject with normal plasma triglycerides.

Conclusion: A compound heterozygous mutation of *LMF1* was identified in the presenting patient with severe HTG. These findings expand on the spectrum of *LMF1* mutations and contribute to the genetic diagnosis and counseling of families with HTG.

See editorial vol. 26: 128-131

Key words: Hypertriglyceridemia, HTG, *LMF1*, Compound mutation

Introduction

Severe hypertriglyceridemia (HTG) is a disorder characterized by elevated plasma triglyceride levels of >885 mg/dL (10.0 mmol/L)[1)] or >1000 mg/dL (11.2 mmol/L)[2)] and could lead to chylomicronemia syndrome. The clinical features of HTG include recurrent episodes of pancreatitis, abdominal pain with nausea and vomiting, hepatosplenomegaly, lipemia retinalis, and eruptive xanthomata[3-5)]. It is also one of the most critical risk factors of atherosclerosis that can result in cerebrovascular[6)] and cardiovascular diseases[7, 8)].

Address for correspondence: Xinjiang Zhang, Department of Neurology, Affiliated Hospital of Yangzhou University, Yangzhou 225000, China
E-mail: lzhangxinjiang@sina.com

Received: February 20, 2018 Accepted for publication: April 24, 2018

Severe HTG is mainly divided into primary and secondary types. The common causes of secondary HTG are renal failure, nephrotic syndrome, diabetes mellitus, hypothyroidism, excessive alcohol or drug use, and some rare endocrine and metabolic disorders[9, 10]. Primary or genetic HTG with a monogenic etiology is less common and is characterized by the accumulation of TG-rich lipoproteins (chylomicrons, very low–density lipoprotein, and remnant lipoproteins) in the plasma[11-13]. These patients are usually carriers of homozygous or compound heterozygous loss-of-function mutations of genes for HTG, including *LPL*, *APOC2*, *APOA5*, *GPIHBP1*, *LMF1*, and *GPD1*.

LPL, a principal enzyme in plasma lipid metabolism, is synthesized in the endoplasmic reticulum (ER) of parenchymal cells in muscle and adipose tissue[14, 15]. It is the rate-limiting enzyme in tissues that hydrolyzes triglycerides in chylomicrons and very low–density lipoprotein (VLDL) and thereby makes fatty acids available for tissue metabolism[15]. Meanwhile, macrophage foam cell formation is associated with triglyceride-rich lipoprotein (TGRL)-LPL-VLDL receptor pathway, which is a critical step in the initial stages of atherosclerotic process[16]. As a result, the activity of LPL is a key determinant of tissue lipid partitioning and plasma TG clearance. LPL deficiency results in elevated plasma TGs[17]. In adipocytes, lipase maturation is the conversion of the newly synthesized LPL into a catalytically active enzyme, which involves glycosylation, glycan processing, folding, and the assembly of homodimers[18]. *LMF1* encodes a transmembrane protein chaperone localized to the ER membrane that is involved in the maturation of LPL and hepatic lipase (HL)[19, 20]. Patients with deleterious mutation in *LMF1* also show combined lipase deficiency (cld) with concomitant HTG and associated disorders[17].

According to the Human Gene Mutation Database, there are only 14 mutations that have been identified in previous mutation screenings of *LMF1* in patients with familial or sporadic HTG. In the present study, a compound heterozygous mutation (c.257C>T/p.P86L and c.1184C>T/p.T395I) in exons 2 and 8 of *LMF1* was identified and co-segregated with the affected patient in this family. Both mutations were first reported worldwide. Bioinformatics analysis predicted that both mutations were deleterious. Neither of them has been presented in the single nucleotide polymorphism (dbSNP) databases (https://www.ncbi.nlm.nih.gov/projects/SNP/) and Exome Variant Server databases (http://evs.gs.washington.edu/EVS/).

Materials and Methods

Patients

In this study, a family of seven members from Jiangsu province across three generations, which was admitted to the Affiliated Hospital of Yangzhou University, 2017 (Yangzhou, China), was included (**Fig. 1A**, **Table 1**). The proband was diagnosed with severe HTG with a plasma triglyceride level of 38.70 mmol/L. No severe HTG was present in the other family members. The present study was approved by the Ethics Committee of Yangzhou University (Yangzhou, China). All subjects provided consent prior to the commencement of the study.

DNA Extraction

Genomic DNA was extracted from the peripheral blood of the proband and other family members by using a DNeasy Blood and Tissue kit (Qiagen, Inc., Valencia, CA, USA) according to the manufacturer's protocol, on the QIAcube automated DNA extraction robot (Qiagen, Inc.).

Mutation Sequencing

Several genes were amplified by polymerase chain reaction (PCR), including *LMF1* (Refseq: NM_022773), *LPL* (Refseq: NM_000237), *APOC2* (Refseq: NM_001646), *APOA5* (Refseq: NM_052968), *GPD1* (Refseq: NM_005276), and *GPIHBP1* (Refseq: NM_178172). PCR was performed using 25-μL reaction volumes, containing 0.3-mM dNTPs, 1X PCR buffer (10-mM Tris-HCl, pH 9.0; 50-mM KCl; 0.1% Triton X-100; and 0.01% w/v gelatin), 2.0-mM MgCl2, 0.5 μM of each primer, 1.5 U of Taq polymerase (Thermo Fisher Scientific, Inc., Waltham, MA, USA), and 50 ng of genomic DNA. Thermocycling conditions were as follows: Initial denaturation at 95℃ for 5 min, followed by 35 cycles of amplification consisting of denaturation at 95℃ for 30 sec, annealing at 55℃–61℃ for 30 sec, and extension at 72℃ for 1 min. A final extension step was performed at 72℃ for 7 min. The sequences of the PCR products were obtained using the ABI 3100 genetic analyzer (Applied Biosystems; Thermo Fisher Scientific, Inc.). The primers are supplied in **Supplementary Table 1**.

Bioinformatics Analysis and Mutation Prediction

Multiple *LMF1* protein sequences were aligned in several species (version 3.6; http://www.ncbi.nlm.nih.gov). Polymorphism Phenotyping-2[21] , Sorting Intolerant From Tolerant[22], and MutationTaster[23] were used to predict the effects of these sequence variants on the function of LMF1 protein.

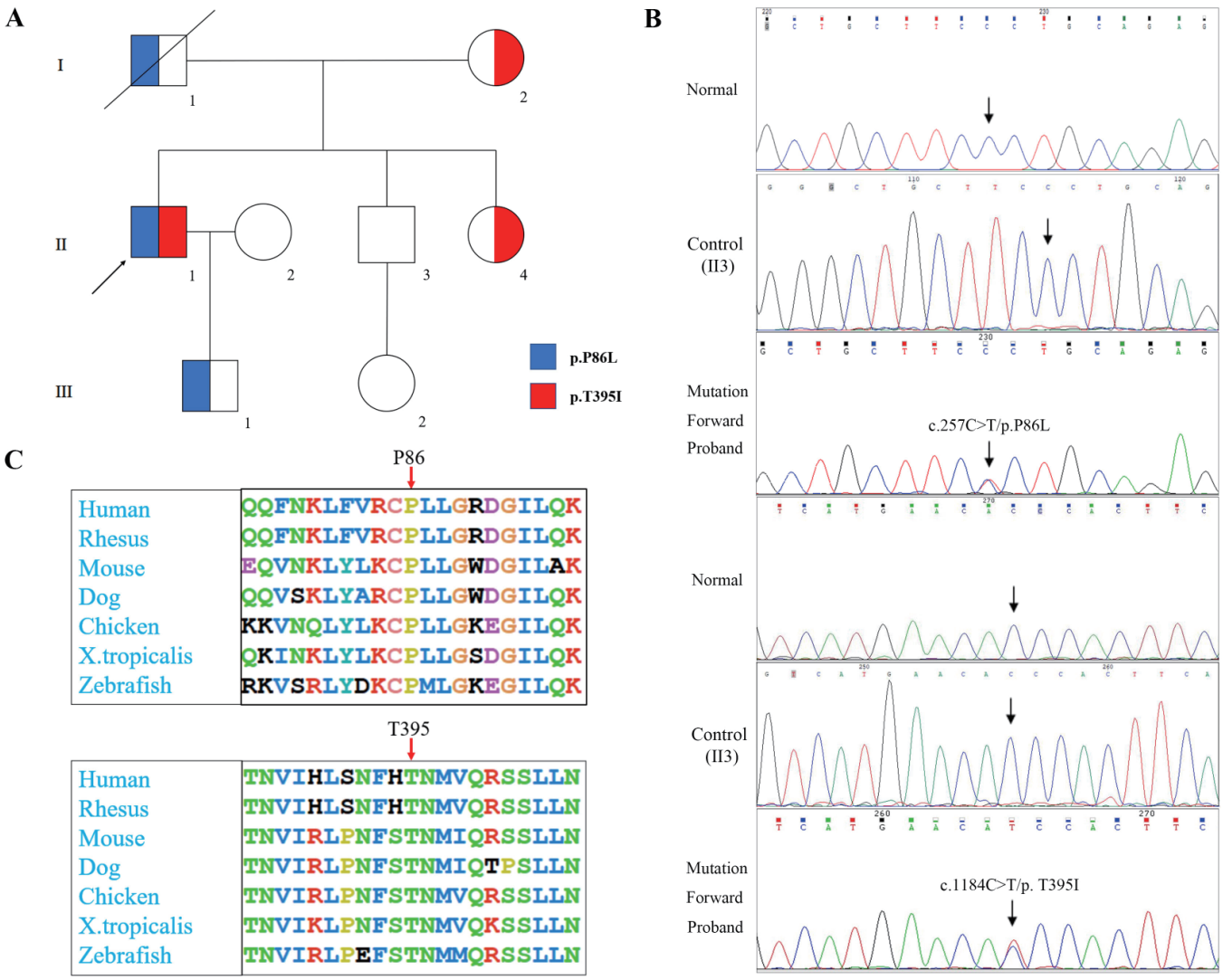

Fig. 1. Pedigree and genetic analysis of the family with HTG

(A) Pedigree of the family. Red and blue circles/squares are affected, white circles/squares are unaffected. Arrow indicates the proband. (B) Sanger DNA sequencing chromatogram demonstrates the heterozygosity for the mutations (c.257C>T and c.1184C>T) of *LMF1*. (C) Conservation analysis of *LMF1*. P86 and T395 sites are highlighted in red. This locus was found to be highly conserved at the protein level across different species.

LPL and HL Assays

We measured the pre-heparin and post-heparin LPL and HL activity. Blood samples were collected from the proband and his brother (II-3) after an overnight fast. Their blood was collected again 15 min after an intravenous bolus injection of heparin (100 IU/kg). His brother was set as a normal control for triglyceride. LPL and HL activity assays of other family members were not performed because they refused to receive heparin. LPL and HL activities were measured as previously described[24]. LPL and HL activities were detected by LPL Activity Assay Kit (Sigma, Roar Biomedical Inc.). Each assay was performed in three independent tests. The data were analyzed by unpaired two-tailed tests using Graph Pad Prism V.5 software (V.5.0).

Result

Clinical Case

We reported a case of a 51-year-old man who was hospitalized for mild left-sided hemiplegia (**Fig.1A**, **Table 1**). Magnetic resonance imaging confirmed the diagnosis of lacunar infarction. Lipid profile assessment revealed the presence of HTG (TG, 38.70 mmol/L). His body mass index was 24.7 kg/m^2.

Table 1. Summary of the family with severe HTG investigated

Family member	TG (mmol/L)	Age (years)	*LMF1* mutations		Program prediction		
			DNA	Protein	Polyphen-2	SIFT	MutationTaster
II1 (proband)	38.7	51	257C>T	P86L	Probably Damaging (0.998)	Damaging (0.003)	Disease Causing (0.999)
			1184C>T	T395I	Possibly Damaging (0.591)	Damaging (0.013)	Disease Causing (0.642)
II	N/A	70	N/A	N/A	N/A	N/A	N/A
I2	3.22	69	1184C>T	T395I	Possibly Damaging (0.591)	Damaging (0.013)	Disease Causing (0.642)
II2	1.52	50	Normal	Normal	N/A	N/A	N/A
II3	1.44	47	Normal	Normal	N/A	N/A	N/A
II4	2.99	45	1184C>T	T395I	Possibly Damaging (0.591)	Damaging (0.013)	Disease Causing (0.642)
III1	3.07	22	257C>T	P86L	Probably Damaging (0.998)	Damaging (0.003)	Disease Causing (0.999)
III2	1.52	20	Normal	Normal	N/A	N/A	N/A

HTG, hypertriglyceridemia; TG, triglyceride; *LMF1*, lipase maturation factor 1; N/A, not available.

He had a history of pancreatitis, but did not have a history of diabetes, hypertension, smoking, or alcohol abuse. However, he had a family history of cardiovascular disease: His father had died of coronary heart disease at the age of 70 years. Secondary causes of severe HTG were ruled out. An impaired glucose tolerance was indicated by an HbA1c of 5.6%. Doppler ultrasound showed that he had atherosclerotic changes with plaques in the right internal carotid artery. Diffusion-weighted imaging showed lacunar infarction of right basal ganglia, while magnetic resonance angiography showed multiple intracranial vascular stenosis. Another three family members showed the HTG phenotype, but the levels of TG were not very high (**Table 1**).

Molecular Genetic Analysis

The possibility of HTG induction by known genes was investigated. Two missense mutations (c.257C>T/p.P86L and c.1184C>T/p.T395I) in *LMF1* were identified by using Sanger sequencing, and they were co-segregated with the affected members (**Fig. 1B**). The allelic segregation analysis revealed that the c.1184C>T/p.T395I mutation was carried by the mother, whereas the c.257C>T/p.P86L mutation was speculated to be inherited from the father. These two novel mutations (c.257C>T/p.P86L and c.1184C>T/p.T395I) caused a substitution of proline by leucine and a substitution of threonine by isoleucine in *LMF1*. The maximum frequency values (MAF) of both mutations in Exome Aggregation Consortium and TGP (1000 genomes project) database were less than 0.001. Both mutations were not found in our 200 control cohorts, as well as in dbSNP and Exome Variant Server database. The amino acid sequences of *LMF1* were found to be aligned in humans, rhesus monkeys, mice, dogs, chicken, and zebrafishes, which revealed that the affected amino acids were evolutionarily conserved (**Fig. 1C**). The two variants were probably damaging (c.257C>T/p.P86L) and possibly damaging (c.1184C>T/p.T395I) in Polyphen-2, were both damaging in SIFT, and were disease causing in MutationTaste. The uniformity among the detrimental effects of the variants as predicted by all these bioinformatics programs suggested that these mutations are important in the function of LMF1.

LPL and HL Activities

We measured the post-heparin LPL and HL activities of the proband and a normal plasma triglyceride family member (II3) as a control. The levels of the plasma post-heparin LPL and HL activities in the proband (57 and 177 mU/mL) were reduced to 24% and 75%, respectively, compared with those assayed in the normal plasma triglyceride control (**Fig. 2**).

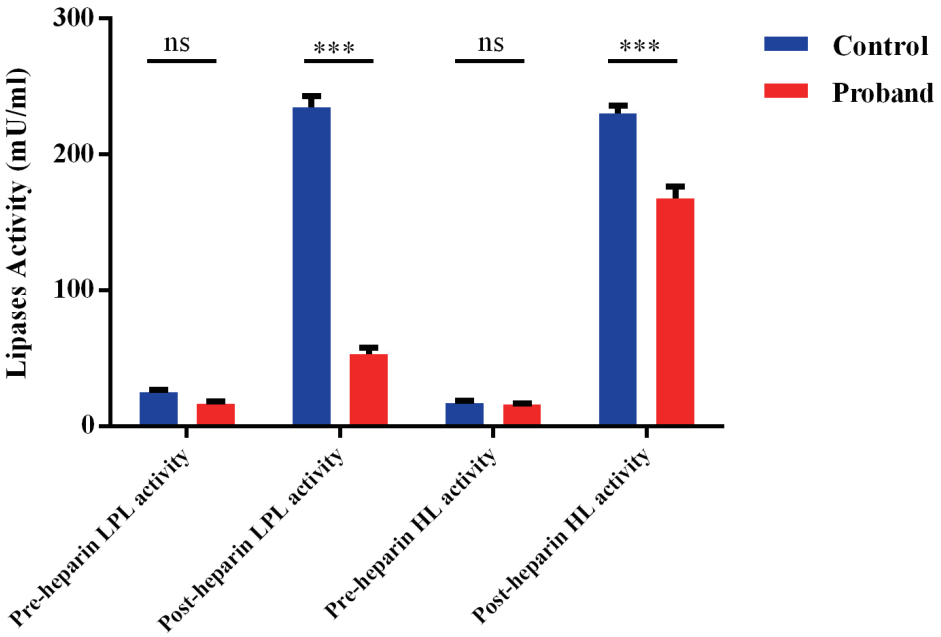

Fig. 2. Pre-heparin and post-heparin LPL and HL activity were assayed in the proband and a normal plasma triglyceride control

The levels of the plasma post-heparin LPL and HL activity in the proband (57 and 177 mU/mL) were reduced to 24% and 75%, respectively, compared with those assayed in the normal plasma triglyceride control.

Discussion

In this study, we report a severe HTG patient with mild stroke associated with a compound heterozygous mutation (c.257C>T/p.P86L and c.1184C>T/p.T395I) in exons 2 and 8 of *LMF1*. Our study is consistent with the previous reports that only homozygous and compound heterozygous mutations in *LMF1* are responsible for HTG[17, 25]. At the same time, we found that the LPL and HL activities were lower in the proband than in the healthy subject. At present, 14 point mutations of *LMF1* have been reported in HTG patients (**Fig. 3A**). The outcome of the molecular genetic investigations performed in the present study was consistent with previous studies[17, 25], which indicated that mutations in *LMF1* could cause HTG due to the decreasing activities of LPL and HL.

Combined lipase deficiency (cld) is a recessive mutation located on mouse chromosome 17 that exhibits severely deficient LPL activity[26]. Homozygous cld mutant mice develop extreme HTG as a result of severe deficiency in plasma LPL and HL activities and die within 48 hours or a few days after birth[26, 27]. The precise genetic location of *LMF1*, which encodes an ER-resident, transmembrane protein containing cld, has been identified[17]. *LMF1* plays a critical role in the post-translational maturation of LPL and HL. Deleterious mutations in *LMF1* gene result in severe HTG[17, 25]. Our study also demonstrated that lipase activities, especially the LPL activity, in the proband decreased significantly.

Previous studies revealed that LMF1 is a multipass transmembrane protein of the ER that has five transmembrane domains, with its three soluble domains and C-terminus in the ER lumen and N-terminus in the cytosol. The loops (labeled A–D) connect all these domains (**Fig. 3B**). This subcellular structure is consistent with the post-translational effect of *LMF1* for the maturation of lipases, as these enzymes attain catalytic activity within the ER[28, 29]. It is also demonstrated that the loop C and the C-terminus of *LMF1* are important for dimeric lipase maturation[30]. Indeed, two nonsense mutations (Y439X and W464X) in the C-terminal ER domain of *LMF1* resulted in truncated variants[17, 25]. In both cases, severe HTG were associated with deficiency for lipase maturation. In the present study, these two mutations are both located in the ER lumen. The novel mutation (c.257C>T/p.P86L) located in loop-A domain resulted in the substitution of proline by leucine. The loop-A domain of *LMF1* is a part of lipase-binding domain and plays a crucial role in activating lipase activity[19, 30, 31]. Another novel mutation (c.1184C>T/p.T395I) located in the highly conserved C-termi-

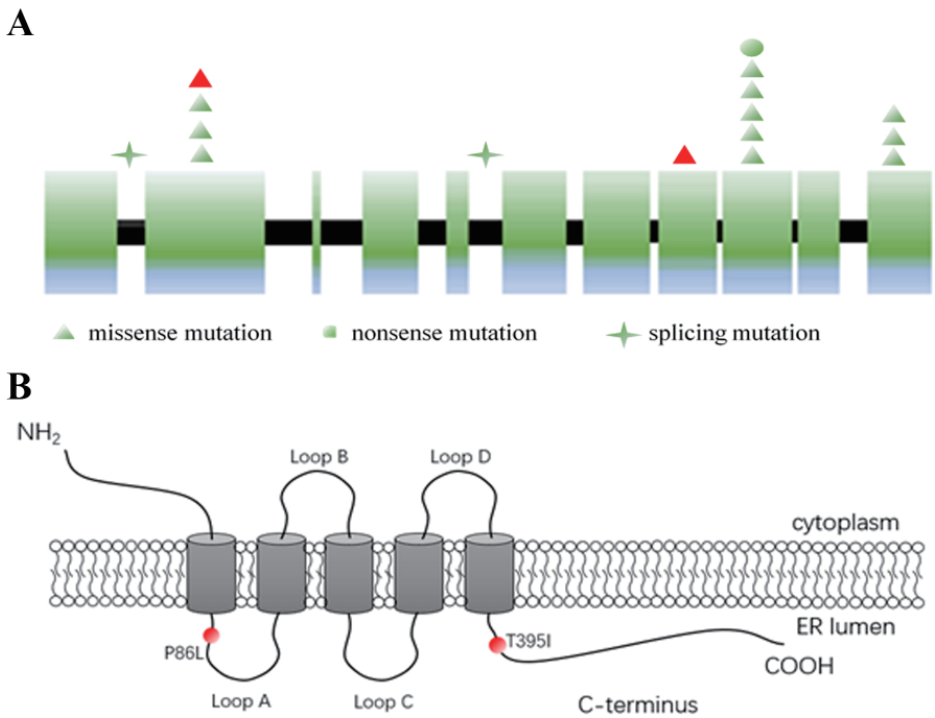

Fig. 3. Summary of mutation Structure analysis of *LMF1*

(A) Summary of missense, nonsense, or splicing mutations in *LMF1* associated with hypertriglyceridemia. Mutations identified in the present study are indicated in red. (B) Schematic of *LMF1*'s topology. Mutation (c.257C>T/p.P86L) located in loop-A and the other mutation (c.1184C>T/p.T395I) located in the C-terminus domain are indicated in red.

nus domain, which is also part of the lipase maturation domain, contributes to the maturation of lipase. Consequently, they may both affect the function of *LMF1*, further down-regulate the activities of LPL and HL and lead to HTG.

Previous studies have confirmed that nonsense mutation of *LMF1* affects the activity of lipoprotein lipase and can cause HTG[17, 25]. Patients carrying p.Y439X mutation showed severe HTG, recurrent episodes of pancreatitis, and diabetes mellitus. However, another patient harboring p.W464X mutation only presented HTG[17]. The differences indicated that mutations in *LMF1* may show high genetic heterogeneity and complexity in a diverse genetic architecture[32, 33].

In-depth diagnostic strategies of HTG are not routinely used in clinical works due to the costs of genetic analysis and the low prevalence of severe HTG. In this study, we have shown that a molecular diagnosis is feasible and effective in carefully selected patients with severe HTG. Fortunately, a highly accurate customized generation sequencing (NGS) panel for targeted gene sequencing of 18 genes involved in HTG has been recently developed, which offers the possibility to perform massively parallel sequencing that can produce enormous amounts of data and allow analysis of genes in a short period of time, easily and relatively economically[34]. Differential therapeutic strategies may be developed by unraveling the underlying molecular "defect." In the last decades, different types of vectors have been used in clinical trials for treating several monogenetic disorders. A recent study showed that *LPL* gene therapy had been used successfully in patients with genetic LPL deficiency, and the follow-up study is ongoing to definitively confirm the long-term benefit and safety of this approach[35]. However, there are currently no clear treatment options for *LMF1* deficiency. Currently, only one research has reported that fibrate drug combination therapy + insulin may decrease the level of TG in patients with *LMF1* mutation[25]. Fibrates are widely used clinically to treat HTG. They activate peroxisome proliferator-activated receptors (PPARs), especially PPARα. PPARs can modulate fat metabolism and adipose tissue differentiation. They can further upregulate the transcription of multiple genes that facilitate lipid metabolism, such as apolipoprotein (apo) A-5 and LPL, and downregulate the transcription of apoC-3. However, fibrates have less selectivity for PPARα and are associated with a high risk of side effects[36]. Recently, K-877

(Pemafibrate) has been shown to have higher PPARα agonistic activity and selectivity than fibrates[37]. In a recent study, Sairyo *et al.* used K-877 to treat a mouse model with diet-induced HTG. The result revealed that K-877 and fenofibrate could both increase the activity of LPL and decrease the level of serum TG. Moreover, K-877 may attenuate postprandial HTG more effectively than fenofibrate by suppressing the postprandial increase of chylomicrons and the accumulation of chylomicron remnants[38]. In our subsequent work, we plan to treat our patient with K-877. We hope this measure can decrease the TG level of the proband and provide more acute therapy examples for clinic.

In conclusion, the present study identified an *LMF1* compound heterozygous mutation (c.257C>T/p.P86L and c.1184C>T/p.T395I) in a patient with severe HTG. To the best of our knowledge, this is the first report of these two mutations worldwide. The results of the present study offer further support for the significant involvement of *LMF1* in severe HTG. The results also expand on the spectrum of *LMF1* mutations and contribute to the genetic diagnosis and counseling of families with severe HTG.

Acknowledgments

We thank the patients and their families for participating in this study. This study was supported by Guided research projects of Jiangsu Department of Health (Z201014).

Conflict of Interest

None.

References

1) de Graaf J, Couture P, Sniderman A: A diagnostic algorithm for the atherogenic apolipoprotein B dyslipoproteinemias. Nat Clin Pract Endocrinol Metab, 2008; 4: 608-618
2) Talmud PJ, Smart M, Presswood E, Cooper JA, Nicaud V, Drenos F, Palmen J, Marmot MG, Boekholdt SM, Wareham NJ, Khaw KT, Kumari M, Humphries SE: ANGPTL4 E40K and T266M: effects on plasma triglyceride and HDL levels, postprandial responses, and CHD risk. Arterioscler Thromb Vasc Biol, 2008; 28: 2319-2325
3) Fellin R, Baggio G, Poli A, Augustin J, Baiocchi MR, Baldo G, Sinigaglia M, Greten H, Crepaldi G: Familial lipoprotein lipase and apolipoprotein C-II deficiency. Lipoprotein and apoprotein analysis, adipose tissue and hepatic lipoprotein lipase levels in seven patients and their first degree relatives. Atherosclerosis, 1983; 49: 55-68
4) Avis HJ, Scheffer HJ, Kastelein JJ, Dallinga-Thie GM, Wijburg FA: Pink-creamy whole blood in a 3-month-old infant with a homozygous deletion in the lipoprotein lipase gene. Clin Genet, 2010; 77: 430-433
5) Rahalkar AR, Hegele RA: Monogenic pediatric dyslipidemias: classification, genetics and clinical spectrum. Mol Genet Metab, 2008; 93: 282-294
6) Antonios N, Angiolillo DJ, Silliman S: Hypertriglyceridemia and ischemic stroke. Eur Neurol, 2008; 60: 269-278
7) Murad MH, Hazem A, Coto-Yglesias F, Dzyubak S, Gupta S, Bancos I, Lane MA, Erwin PJ, Berglund L, Elraiyah T, Montori VM: The association of hypertriglyceridemia with cardiovascular events and pancreatitis: a systematic review and meta-analysis. BMC Endocr Disord, 2012; 12: 2
8) Okamoto Y, Tominaga K, Uemura S, Matsuoka H, Tsujii T, Nakano H: Hypertriglyceridemia caused by the autoantibody to lipases for plasma lipoproteins: a case report. J Atheroscler Thromb, 1995; 2: 66-69
9) Brunzell JD: Clinical practice. Hypertriglyceridemia. N Engl J Med, 2007; 357: 1009-1017
10) Yuan G, Al-Shali KZ, Hegele RA: Hypertriglyceridemia: its etiology, effects and treatment. CMAJ, 2007; 176: 1113-1120
11) Talmud PJ, Hawe E, Martin S, Olivier M, Miller GJ, Rubin EM, Pennacchio LA, Humphries SE: Relative contribution of variation within the APOC3/A4/A5 gene cluster in determining plasma triglycerides. Hum Mol Genet, 2002; 11: 3039-3046
12) Vaxillaire M, Cavalcanti-Proença C, Dechaume A, Tichet J, Marre M, Balkau B, Froguel P: The common P446L polymorphism in GCKR inversely modulates fasting glucose and triglyceride levels and reduces type 2 diabetes risk in the DESIR prospective general French population. Diabetes, 2008; 57: 2253-2257
13) Charrière S, Peretti N, Bernard S, Di FM, Sassolas A, Merlin M, Delay M, Debard C, Lefai E, Lachaux A, Moulin P, Marçais C: GPIHBP1 C89F neomutation and hydrophobic C-terminal domain G175R mutation in two pedigrees with severe hyperchylomicronemia. J Clin Endocrinol Metab, 2011; 96: E1675-1679
14) Doolittle MH, Péterfy M: Mechanisms of lipase maturation. Clin Lipidol, 2010; 5: 71-85
15) Wang H, Eckel RH: Lipoprotein lipase: from gene to obesity. Am J Physiol Endocrinol Metab, 2009; 297: E271-288
16) Takahashi S: Triglyceride Rich Lipoprotein -LPL-VLDL Receptor and Lp(a)-VLDL Receptor Pathways for Macrophage Foam Cell Formation. J Atheroscler Thromb, 2017; 24: 552-559
17) Péterfy M, Ben-Zeev O, Mao HZ, Weissglas-Volkov D, Aouizerat BE, Pullinger CR, Frost PH, Kane JP, Malloy MJ, Reue K, Pajukanta P, Doolittle MH: Mutations in LMF1 cause combined lipase deficiency and severe hypertriglyceridemia. Nat Genet, 2007; 39: 1483-1487
18) Pajukanta P, Terwilliger JD, Perola M, Hiekkalinna T, Nuotio I, Ellonen P, Parkkonen M, Hartiala J, Ylitalo K, Pihlajamäki J, Porkka K, Laakso M, Viikari J, Ehnholm C, Taskinen MR, Peltonen L: Genomewide scan for familial combined hyperlipidemia genes in finnish families, suggesting multiple susceptibility loci influencing triglyceride, cholesterol, and apolipoprotein B levels. Am J Hum Genet, 1999; 64: 1453-1463

19) Doolittle MH, Ehrhardt N, Péterfy M: Lipase maturation factor 1: structure and role in lipase folding and assembly. Curr Opin Lipidol, 2010; 21: 198-203
20) Péterfy M: Lipase maturation factor 1: a lipase chaperone involved in lipid metabolism. Biochim Biophys Acta, 2012; 1821: 790-794
21) Sunyaev S, Ramensky V, Bork P: Towards a structural basis of human non-synonymous single nucleotide polymorphisms. Trends Genet, 2000; 16: 198-200
22) Ng PC, Henikoff S: SIFT: Predicting amino acid changes that affect protein function. Nucleic Acids Res, 2003; 31: 3812-3814
23) Schwarz JM, Rödelsperger C, Schuelke M, Seelow D: MutationTaster evaluates disease-causing potential of sequence alterations. Nat Methods, 2010; 7: 575-576
24) Huttunen JK, Ehnholm C, Kinnunen PK, Nikkilä EA: An immunochemical method for the selective measurement of two triglyceride lipases in human postheparin plasma. Clin Chim Acta, 1975; 63: 335-347
25) Cefalù AB, Noto D, Arpi ML, Yin F, Spina R, Hilden H, Barbagallo CM, Carroccio A, Tarugi P, Squatrito S, Vigneri R, Taskinen MR, Péterfy M, Averna MR: Novel LMF1 nonsense mutation in a patient with severe hypertriglyceridemia. J Clin Endocrinol Metab, 2009; 94: 4584-4590
26) Paterniti JR, Brown WV, Ginsberg HN, Artzt K: Combined lipase deficiency (cld): a lethal mutation on chromosome 17 of the mouse. Science, 1983; 221: 167-169
27) Coleman T, Seip RL, Gimble JM, Lee D, Maeda N, Semenkovich CF: COOH-terminal disruption of lipoprotein lipase in mice is lethal in homozygotes, but heterozygotes have elevated triglycerides and impaired enzyme activity. J Biol Chem, 1995; 270: 12518-12525
28) Ben-Zeev O, Mao HZ, Doolittle MH: Maturation of lipoprotein lipase in the endoplasmic reticulum. Concurrent formation of functional dimers and inactive aggregates. J Biol Chem, 2002; 277: 10727-10738
29) Ben-Zeev O, Doolittle MH: Maturation of hepatic lipase. Formation of functional enzyme in the endoplasmic reticulum is the rate-limiting step in its secretion. J Biol Chem, 2004; 279: 6171-6181
30) Doolittle MH, Neher SB, Ben-Zeev O, Ling-Liao J, Gallagher CM, Hosseini M, Yin F, Wong H, Walter P, Péterfy M: Lipase maturation factor LMF1, membrane topology and interaction with lipase proteins in the endoplasmic reticulum. J Biol Chem, 2009; 284: 33623-33633
31) Babilonia-Rosa MA, Neher SB: Purification, cellular levels, and functional domains of lipase maturation factor 1. Biochem Biophys Res Commun, 2014; 450: 423-428
32) Johansen CT, Hegele RA: The complex genetic basis of plasma triglycerides. Curr Atheroscler Rep, 2012; 14: 227-234
33) Tune JD, Goodwill AG, Sassoon DJ, Mather KJ: Cardiovascular consequences of metabolic syndrome. Transl Res, 2017; 183: 57-70
34) Cefalù AB, Spina R, Noto D, Ingrassia V, Valenti V, Giammanco A, Fayer F, Misiano G, Cocorullo G, Scrimali C, Palesano O, Altieri GI, Ganci A, Barbagallo CM, Averna MR: Identification of a novel LMF1 nonsense mutation responsible for severe hypertriglyceridemia by targeted next-generation sequencing. J Clin Lipidol, 2017; 11: 272-281. e8
35) Stroes ES, Nierman MC, Meulenberg JJ, Franssen R, Twisk J, Henny CP, Maas MM, Zwinderman AH, Ross C, Aronica E, High KA, Levi MM, Hayden MR, Kastelein JJ, Kuivenhoven JA: Intramuscular administration of AAV1-lipoprotein lipase S447X lowers triglycerides in lipoprotein lipase-deficient patients. Arterioscler Thromb Vasc Biol, 2008; 28: 2303-2304
36) Fruchart JC: Selective peroxisome proliferator-activated receptor α modulators (SPPARMα): the next generation of peroxisome proliferator-activated receptor α-agonists. Cardiovasc Diabetol, 2013; 12: 82
37) Yamazaki Y, Abe K, Toma T, Nishikawa M, Ozawa H, Okuda A, Araki T, Oda S, Inoue K, Shibuya K, Staels B, Fruchart JC: Design and synthesis of highly potent and selective human peroxisome proliferator-activated receptor alpha agonists. Bioorg Med Chem Lett, 2007; 17: 4689-4693
38) Sairyo M, Kobayashi T, Masuda D, Kanno K, Zhu Y, Okada T, Koseki M, Ohama T, Nishida M, Sakata Y, Yamashita S: A Novel Selective PPARα Modulator (SPPARMα), K-877 (Pemafibrate), Attenuates Postprandial Hypertriglyceridemia in Mice. J Atheroscler Thromb, 2018; 25: 142-152

Supplementary Table 1. Primers sequences used for mutation sequencing of LMF1, APOA5, APOC2, GPIHBP1, LPL and GPD1 genes

Gene	Forward 5' → 3'	Reverse 5' → 3'
LMF1-1	AACTGCGAAGGAGGCAGGC	GCGGAGGAGTCTCGAGGGAG
LMF1-2	TGCCTCGCCCCGCATTCT	AGCTCCGACCGCCCCATT
LMF1-3	GGTTGAAACAAGCCAAAGTGT	AGATCACAAGCGCCCATC
LMF1-4	CTTGCGTGTCGATGTTGA	GGTTAGAAGAGCCACCGTTA
LMF1-5	CCCTTCTCAAATTCTGCCTTCC	TGATGCGACAGCTCACCAG
LMF1-6	CTCTTAGCGTGGCAGGTGG	CAAACGAAGGCTGGGGAG
LMF1-7	GGCACAGCTGGGTTTCA	TGAGCCACCTACCGAATCT
LMF1-8	GCGTGCCAGGAACAAGGT	TGTCCAGGCCCGGTAGTG
LMF1-9	GGGCCACAGTTCCCAAA	CGTTCTAGAAACCTGCCATCTAT
LMF1-10	GAACCCACCTCCAGGAAAG	TGATGCCAAGGCTGATGT
LMF1-11	TTGCTGCGCTGTTCACT	GCTGGGTCTTCGCCTTTATT
APOA5-1	TGTCCCTTCGTCTCCTTCTT	CTGTGGAGAGGGACTAGGTAAT
APOA5-2	CTGATTACCTAGTCCCTCTCCA	AACAGCTACGGAGTTGTCAAG
APOA5-3-1	GGGACAAAGGAGATGATGGA	TCGGCGTATGGGTGGAAG
APOA5-3-2	CTGAAGCCCTACACGATGGA	GCGGAAAGCCTGAAGTCG
APOA5-3-3	GGCACTGGGACTGAGGAAG	GACAAGGAGCTGGGAATGG
APOC2-1	TGGGAAACTTGACTGGGACA	GGCTGGGAAGATGCTTGG
APOC2-2	CCTGGTATTGGGATTTGGT	AACTTCTGGGTCCTGGATG
APOC2-3	CCCCTCCTCCCTCTAACCA	GTGCCATCCATGAGAAGCAA
GPIHBP1-1	ATGCCCTTCATCCCACTTACC	GCTTCCATCCATGCTGCTCT
GPIHBP1-2	GTAGGGTGTTCAGGGTAGGG	CAGAATGCTCCAGGCAGAT
GPIHBP1-3	CTCACCAGGCTAGGCTTTGG	TGGAGTGGGTGGTCAGGAGG
GPIHBP1-4	CGCCCATCCTCAGCACTT	CGCCCAAGACACTCCAAATC
LPL-1	GGAAAGCTGCCCACTTCTA	TTCCTTCTTCTCATCCTCAGTTC
LPL-2	TGGTTGCCTGTGAACCTAAA	CCTGAGCCAGAACTGTCTTTAT
LPL-3	GACAAGTGGTAGGTGGGTATTT	CCACGCTGATTCTGAAGATTTG
LPL-4	GGCAGAACTGTAAGCACCTT	CCTAATAAAGAGCCCTACAATGAGATA
LPL-5	GCCAGTGCATTCAAATGATGAG	TGGGTCAATAAGGGTTAAGGATAAG
LPL-6	ATGCCAAATGAAACACTC	TTAGAAGCCTCAGACAAA
LPL-7	CTTCCGGTTTGAGTGCTAGT	TGCTCAGACCAAGGGTTATG
LPL-8	TGAGTTCTTTGTTGGACA	CTGAAATACAGCCCCTAG
LPL-9	TCCTGACAGAACTGTACCTTTG	GGATGCCCAGTCAGCTTTA
GPD1-1	TCCTTTCCCTGGCTCTGC	CCTCCTACCCACCTCTGTCTT
GPD1-2	GGGACTATTTGTCATGGGAGT	GAGGCACCTGTTGAGTAAGG
GPD1-3	CCAAACAAGCCTTCCTGC	CTCCTTGCTTCACCCACC
GPD1-4	TGGCCTCCTCACAGCAAA	TCCCAGCCTCCTTCACCT
GPD1-5	TAAGCCCAGGAGTTTGAG	CACGGTCTGATGATGAATAA
GPD1-6	GTCACGGCTGATGAAATGA	CAAGCACCTCTACCTGGAT
GPD1-7	TCTGTAGGCATCCAGGTAG	AGATTTGTGGCAGGTTTAG
GPD1-8	GGAGGGTTAGGCAGTGAG	TTTCTGGCAAATGTGGTG