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

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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.

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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) or $>$1000 mg/dL (11.2 mmol/L) and could lead to chylomicronemia syndrome. The clinical features of HTG include recurrent episodes of pancreatitis, abdominal pain with nausea and vomiting, hepatosplenomegaly, lipemia retinalis, and eruptive xanthoma$. It is also one of the most critical risk factors of atherosclerosis that can result in cerebrovascular$ and cardiovascular diseases$. 
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\(^3,10\). 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\(^11\). 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 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.

### 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 (\textbf{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-\(\mu\)L 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 MgCl\(_2\), 0.5 \(\mu\)M of each primer, 1.5 \(\mu\)L of Taq polymerase (Thermo Fisher Scientific, Inc.). The primers are supplied in \textbf{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 (\textsuperscript{22}), Sorting Intolerant From Tolerant\(\textsuperscript{23}\), and MutationTaster\(\textsuperscript{24}\) were used to predict the effects of these sequence variants on the function of LMF1 protein.
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/m².

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*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*. 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. LPL and HL activities were detected by LPL Activity Assay Kit (Sigma, Roar Biomedical Inc.). Each assay was performed in three
leucine 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 MutationTaster. 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.

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          | Probably Damaging  | 257C>T | P86L    | (0.998)    | Damaging (0.003) | Disease Causing (0.999) |
|                 |             |             | 1184C>T        | Possibly Damaging  | 1184C>T | T395I   | (0.591)    | Damaging (0.013) | Disease Causing (0.642) |
| II   | N/A         | 70          | N/A            | N/A                | N/A  | N/A     | N/A        | N/A  | N/A           |
| I2   | 3.22        | 69          | 1184C>T        | Possibly Damaging  | 1184C>T | T395I   | (0.591)    | Damaging (0.013) | Disease Causing (0.642) |
| I2   | 1.52        | 50          | Normal         | N/A                | N/A  | N/A     | N/A        | N/A  | N/A           |
| I3   | 1.44        | 47          | Normal         | N/A                | N/A  | N/A     | N/A        | N/A  | N/A           |
| I4   | 2.99        | 45          | 1184C>T        | Possibly Damaging  | 1184C>T | T395I   | (0.591)    | Damaging (0.013) | Disease Causing (0.642) |
| III1 | 3.07        | 22          | 257C>T         | Probably Damaging  | 257C>T | P86L    | (0.998)    | Damaging (0.003) | Disease Causing (0.999) |
| III2 | 1.52        | 20          | Normal         | N/A                | N/A  | N/A     | 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 MutationTaster. 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).
LPL and HL. Deleterious mutations in LMF1 gene result in severe HTG. Our study also demonstrated that lipase activities, especially the LPL activity, in the proband decreased significantly. Previous studies revealed that LMF1 is a multi-pass 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. It is also demonstrated that the loop C and the C-terminus of LMF1 are important for dimeric lipase maturation. Indeed, two nonsense mutations (Y439X and W464X) in the C-terminal ER domain of LMF1 resulted in truncated variants. 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. Another novel mutation (c.1184C>T/p.T395I) located in the highly conserved C-termi-
that can produce enormous amounts of data and allow analysis of genes in a short period of time, easily and relatively economically34). 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 approach35). 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 PPAR\(\alpha\). 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 PPAR\(\alpha\) and are associated with a high risk of side effects36). Recently, K-877

![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 \(\rightarrow\) T/p.P86L) located in loop-A and the other mutation (c.1184C \(\rightarrow\) 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 \(LMFI\) affects the activity of lipoprotein lipase and can cause HTG17, 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. The differences indicated that mutations in \(LMFI\) may show high genetic heterogeneity and complexity in a diverse genetic architecture32, 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 economically34). 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 approach35). However, there are currently no clear treatment options for \(LMFI\) deficiency. Currently, only one research has reported that fibrate drug combination therapy + insulin may decrease the level of TG in patients with \(LMFI\) mutation25). Fibrates are widely used clinically to treat HTG. They activate peroxisome proliferator-activated receptors (PPARs), especially PPAR\(\alpha\). 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 PPAR\(\alpha\) and are associated with a high risk of side effects36). Recently, K-877
(Pemafibrate) has been shown to have higher PPARα agonistic activity and selectivity than fenofibrate. 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. 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.

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Conflict of Interest

None.

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**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   | AACTGCGAAGGAGGAGGAGG           | GCAGGAGGAGGTCTGCAAGGGAGG       |
| LMF1-2   | TGCCTGCCCAGCGATTCT             | AGCTCGGACCGCCAGG              |
| LMF1-3   | GTTGAACAGAAAGCCAAAGGATG        | AGATCAACAGGCGCGCATC           |
| LMF1-4   | CTTGGGTTGGCTGTGTTGA            | GTGTAAGAGGACGCCAGTTTA         |
| LMF1-5   | CCCCCCTAAATCTCTGTTCCCG        | TGATGGAGGTCGTCAACG            |
| LMF1-6   | CTTCTAGGCTGGCAGGTT            | CAAGGAGGCCTGCGGAGG           |
| LMF1-7   | GGCAAGCCTGGTGTCTCCA           | TGAGGCCACTCCAGGAATCT          |
| LMF1-8   | GGCTGCGAGGAAACCTG             | TGTCAGGGCCCGCTGTG            |
| LMF1-9   | GGCCACAGGTTCACCAA             | CGTTCATGAAACCTCCACTTAT        |
| LMF1-10  | GAAACCCACCTCCAGAAAGGAGG       | TGAFCACAGGCTGATG             |
| LMF1-11  | TTCTGCTGGCTGTGTTCACT          | GCTGAGGTCGCTGCTTTTAAT         |
| APOA5-1  | TGTCCTGCTTGTCCTCTTTT          | CTGGGAGGAGGACTAGGTAAT         |
| APOA5-2  | CGATGTCCTCTAGCCCTCTCCA        | AAGCGTACGGAGGTTGCAAG          |
| APOA5-3-1| GGCAAGAAGGAGGATGAGGAGGAGG    | TCCGGGTAGGTCGAGGG            |
| APOA5-3-2| CTGAGGCCCTACAGGATGGA          | CGGGAAAGGCTGAACTG            |
| APOA5-3-3| GCCACAGCTGGAGCTAGGGAAG        | GACAAAGGAGGCTGGGAAAT         |
| APOC2-1  | TGCCGAGACTGACGTGGGGCA         | GGTGAGGGAAGATGCTGTG           |
| APOC2-2  | CCTGCTATGGTGATTTGGATG         | AAAGCTGCTGGTATCTGAGT          |
| APOC2-3  | CCCTCCTCTCCCTTAACCA          | CTGTCGATCCAGGAGG             |
| GPIHBP1-1| ATGCCCTTCCTACCCCTACC         | GCTCCATCCACTGCTGTCT           |
| GPIHBP1-2| GTAGGGTGTGGTGAGGAGGAGGAGG    | CAGAAAGCTCAGGAGG             |
| GPIHBP1-3| CTCAACGGCTAGGCTTGG           | TGGAGTGTCGTCGTCAGGG         |
| GPIHBP1-4| CCAGGAGCTCAGCTCAGGG         | CGCCCAAGGACACTCCAAATC         |
| LPL-1    | GGAAGCCTGGCCACTCTTTCA         | TTCCCTTCTCCTCCTAGGCTCCTG     |
| LPL-2    | TGTTGGCCTGTAACCTAAGA         | CCTGACGGAAGACTGCTTGTTAAT      |
| LPL-3    | GCAAGTGTAGTGGTGATTTT         | CCAGCCTGATTCTGAAAGAGGT      |
| LPL-4    | GGCAGAAGCTGTAAGCACCCTT       | CTTAATAGAGGCCCTACATGAGATA    |
| LPL-5    | GCCAGTCTGATACAAAATGAGGAGG    | TGGCAGAATAGGGTTAAGGATAAG     |
| LPL-6    | ATGCCCAATGAAACACTC          | TTAGAAGCTCGACAAGGA          |
| LPL-7    | CTTCCGGGTGGTTAGTGCTAGT       | TGCTCAGACCAAAGGGTTATG        |
| LPL-8    | TGAGGTGCTTGTTGGACCA         | CTGAAAATAGCCGCCCTG          |
| LPL-9    | TCCCTGACAAAGTCTGACCCCTTG     | GGATGCCCAGTACTGCTTGA        |
| GPD1-1   | TCCCTTTCCCTGGCTCTGCTG       | CCTCCTACCACCTCTGCTGTTT       |
| GPD1-2   | GGAGCTAATTCTTGATTAGGAGGAGG  | GAGGGCCACTGTTGATGAAAGG       |
| GPD1-3   | CCAAAAAGCTCCTCTTGC           | CTCCCTTGGCTCCTCACC          |
| GPD1-4   | TGGCCTCCTCACGAAAAGGAGGAGG   | TCCACCAGCTCCCTACCC          |
| GPD1-5   | TAAGGGCAGGAGGTTGAGGAGGAGG   | CACGGTCTAGTGAATGAAAA         |
| GPD1-6   | GTGAGGGCTGATGAAATGAGGAGG    | CAGACACCTACTGCTGAGAT        |
| GPD1-7   | TGGTGGAGAGAGGCTGCTG         | AGATTTGGTGCCAGGTTTA         |
| GPD1-8   | GGAGGTTAGGCGAGTGGAGGAGG     | TTCTGCGAATGCGGTTG           |