Case Report

Novel pathogenic variant combination in *LPL* causing familial chylomicronemia syndrome in an Asian family and experimental validation *in vitro*: a case report

Huiping Shi[1,2], **Zhaoyue Wang**[1,2]

[1]MOH Key Lab of Thrombosis and Hemostasis, Jiangsu Institute of Hematology, the First Affiliated Hospital of Soochow University, Suzhou, China; [2]Collaborative Innovation Center of Hematology, Soochow University, Suzhou, China

Correspondence to: Zhaoyue Wang, MD, PhD. MOH Key Lab of Thrombosis and Hemostasis, Jiangsu Institute of Hematology, the First Affiliated Hospital of Soochow University, 188 Shizi Street, Suzhou 215006, China; Collaborative Innovation Center of Hematology, Soochow University, 1 Shizi Street, Suzhou 215006, China. Email: zwang11@sina.com.

Background: Familial chylomicronemia syndrome (FCS) is a rare autosomal recessive disorder, typically caused by biallelic pathogenic variants in the lipoprotein lipase (*LPL*) gene. Lipoprotein lipase, encoded by the *LPL* gene, catalyzes the hydrolysis of triglycerides, and its deficiency or dysfunction can lead to chylomicronemia and potentially fatal recurrent acute pancreatitis.

Case Description: Here, we report an Asian child with FCS due to compound heterozygous *LPL* variants. The 4-year-old patient presented with splenomegaly and severe hypertriglyceridemia, specifically chylomicronemia which resulted in abnormal coagulation measured by a turbidity-based assay. Based on the clinical features and family history, the diagnosis of FCS was suspected, and confirmed by the identification of compound heterozygous variants in the *LPL* gene (c.461A>G; p.His154Arg and c.788T>A; p.Leu263Gln) in the patient, inheriting one from each parent. According to the clinical and genetic findings, the patient was diagnosed with FCS. *In vitro* experimental validation found that the *LPL* p.H154R variant reduced the expression of lipoprotein lipase and decreased its lipolytic activity, while the *LPL* p.L263Q variant mainly impaired its lipolytic activity.

Conclusions: FCS was molecularly diagnosed using whole exome sequencing in the case presented. When interpreting abnormal coagulation profiles measured by turbidity-based assay, the possibility of lipemic blood (or chylomicronemia) should be considered and the presence of this phenomenon might indicate the diagnosis of FCS. *In vitro* experiments showed that the two *LPL* variants impaired lipoprotein lipase expression and/or function making them likely to be pathogenic.

Keywords: *LPL* gene; lipoprotein lipase; novel pathogenic variant; familial chylomicronemia syndrome; case report

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Introduction

Familial chylomicronemia syndrome (FCS) is a rare autosomal recessive genetic disorder, with an estimated prevalence of one per million (1,2). FCS is characterized by aberrantly high triglyceride level (>10 mmol/L) in the blood, specifically chylomicronemia, due to the impaired hydrolysis of triglycerides by lipoprotein lipase (LPL), which could lead to the potentially fatal recurrent acute pancreatitis. Clinically, the patients with FCS could present with varying symptoms including nausea, vomiting, abdominal pain, and different clinical features such as eruptive xanthomas, lipemia retinalis, hepatosplenomegaly, pancreatitis, and failure to thrive (3). Eruptive xanthomas are small erythematous or yellow

papules localized on the extensor surfaces of extremities, buttocks, and the back (4), which serve as a clue for severe hypertriglyceridemia. Histologically, accumulation of lipid-laden macrophages in the skin can be identified in eruptive xanthomas, which were known to be generated by endothelial cell mediated intracellular hydrolysis of internalized chylomicrons, subsequent release of lipids and uptake by skin macrophages (5). In contrast to multifactorial chylomicronemia syndrome, the clinical manifestations tend to appear in early life in FCS patients (6).

There are five causal genes in FCS: lipoprotein lipase (*LPL*), apolipoprotein C2 (*APOC2*), apolipoprotein A5 (*APOA5*), glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1 (*GPIHBP1*), and lipase maturation factor 1 (*LMF1*). Biallelic pathogenic variants in *LPL* are the predominant cause of FCS. *LPL* encodes lipoprotein lipase in various cell types, including adipocytes, skeletal muscle cells and cardiac myocytes. After folding and assembly (i.e., maturation) with the help of LMF1 (7), lipoprotein lipase is released extracellularly and binds to GPIHBP1 expressed on capillary endothelial cells, which shuttles lipoprotein lipase into the capillary lumen (8). There, lipoprotein lipase catalyzes the hydrolysis of triglycerides (9), with the help of two regulators: apolipoprotein C-II (apoC-II) (10) and apolipoprotein A-V (apoA-V) (11) (*Figure 1*). While apoC-II serves as a cofactor for lipoprotein lipase, apoA-V affects plasma triglyceride levels possibly by stabilizing the lipolytic machinery via binding to lipoproteins, endothelial proteoglycans, and lipoprotein lipase (12). We present the following case in accordance with the CARE reporting checklist (available at https://tp.amegroups.com/article/view/10.21037/tp-22-15/rc).

Case presentation

A 4-year-old girl was referred to our Thrombosis and Hemostasis center for follow-up findings of extremely prolonged activated partial thromboplastin time (APTT) value (>120 s), prothrombin time (PT) value (>80 s), and thrombin time (TT) value (>50 s) at another hospital, and incidental findings of splenomegaly and lipemia during routine health examination for kindergarten. The patient had no history of bleeding problems or physical findings of coagulopathy.

Physical examination revealed that her spleen was enlarged with its lower edge palpated 2.5 cm below the left costal margin, and splenomegaly was confirmed by B ultrasound, showing a spleen size of 10.5 cm × 3.5 cm. No obvious abnormality in liver, gallbladder or pancreas was found by B ultrasound examination.

A lipid profile showed significantly increased triglyceride (TG) level of 22.5 mmol/L (reference range: 0–1.7 mmol/L) (*Figure 2A*), and marginally increased level of total cholesterol (5.44 mmol/L, reference range: 0–5.2 mmol/L). Levels of HDL-C, LDL-C, and apolipoprotein A-I were slightly decreased, while apolipoprotein B level was normal (*Table 1*). Patient's liver function was normal, except for marginally increased ALT (*Table 1*).

Because chylomicronemia can increase plasma turbidity and interfere with coagulation assay using optical analyzer (13), a repeat coagulation assay was performed using magnetic bead method instead. The results showed a normal coagulation profile: APTT 38.6 s (reference range 28–40 s), PT 14.1 s (reference range 11–14.5s), TT 13.9 s (reference range 14–21s), fibrinogen: 1.96 g/L (reference range 2–5 g/L).

A detailed family history revealed that the patient's father, elder sister, and grandfather also had hypertriglyceridemia (father: TG 7.78 mmol/L; sister: TG 4.2 mmol/L; maternal grandfather: TG 5.4 mmol/L) (*Figure 2B*). The great grandmother had a medical history of diabetes mellitus. The family pedigree could represent a pedigree of an autosomal recessive disorder of hypertriglyceridemia.

Therefore, whole exome sequencing was performed using samples from the patient, her father, mother, elder sister and grandfather. The results showed that the patient had compound heterozygous pathogenic variants in the *LPL* gene: NM_000237.2, c.461A>G (p.H154R, rs1563574212) and c.788T>A (p.L263Q). Both variants have not been functionally characterized before. The *LPL* p.H154R variant was inherited from patient's father, and it was also identified in her elder sister. The *LPL* p.L263Q variant was inherited from patient's mother and found in her grandfather. Sanger sequencing was performed to validate the findings (*Figure 2C*). During the disease course, the patient had no eruptive xanthomas, lipemia retinalis, abdominal pain, nausea, or vomiting. As chylomicronemia increases the risk of recurrent acute pancreatitis, the patient was switched to strict low-fat diet during hospital stay, in which fat was kept below 15% of her daily energy take. The strict low-fat diet helped to reduce TG levels and the lowest level observed during the disease course was 4.91 mmol/L. After the patient was discharged, her TG levels increased due to the altered diet at home. She was administered daily fenofibrate (100 mg), which failed to affect the high TG levels, so the therapy was discontinued.

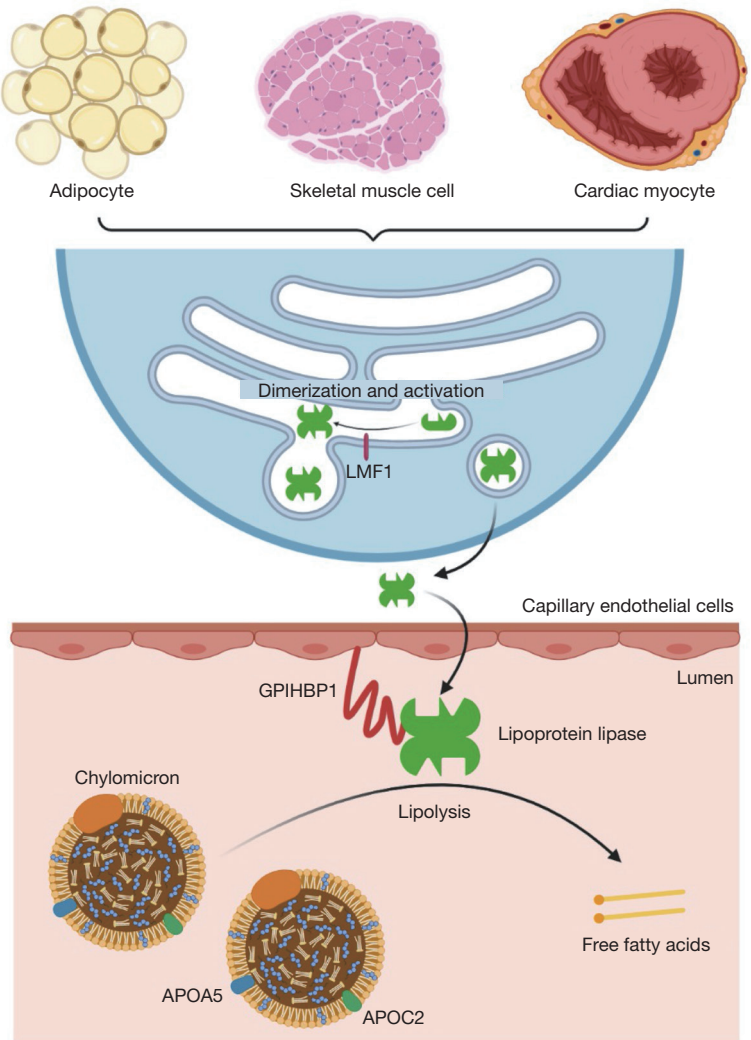
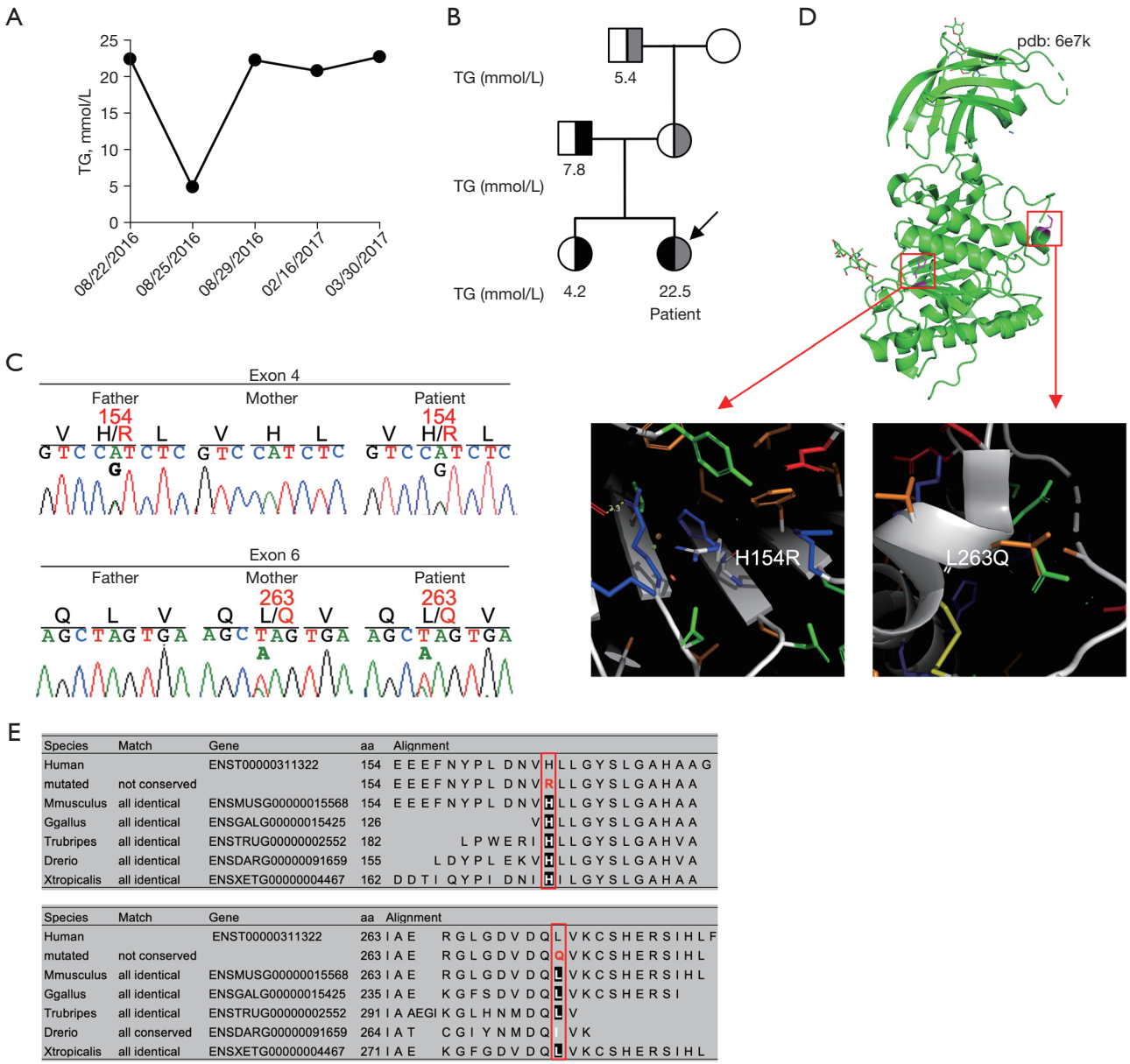


Figure 1 Lipoprotein lipase catalyzes the hydrolysis of triglycerides. Lipoprotein lipase is synthesized in adipocytes, skeletal muscle cells and cardiac myocytes. After dimerization and activation with the help of lipase maturation factor 1 (LMF1), lipoprotein lipase is released extracellularly and binds to glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1 (GPIHBP1) expressed on capillary endothelial cells, which shuttles lipoprotein lipase into the capillary lumen. There, lipoprotein lipase catalyzes the hydrolysis of triglycerides, with the help of two regulators: apolipoprotein C-II and apolipoprotein A-V. ApoC-II serves as a cofactor for lipoprotein lipase, and apoA-V affects plasma triglyceride levels possibly by stabilizing the lipolytic machinery via binding to lipoproteins, endothelial proteoglycans, and lipoprotein lipase. Figure is created with BioRender.

To predict the potential effects of these variants on the function of LPL protein, we downloaded the crystal structure of LPL from the protein data bank (PDB), the code of which is PDB: 6e7k (2). The localizations of the two variants in the LPL protein were visualized in *Figure 2D* using the pymol software. Interestingly, the p.H154R variant was two residues away from Asp-156, which makes the catalytic triad together with Ser-132 and His-241 (14). Homologous protein alignment using MutationTaster2 (15) showed that both His-154 and Leu-263 were conserved among different species (*Figure 2E*), indicating the potential harmful effects of the two *LPL* pathogenic variants. The pathogenicity of these two variants was predicted using PANTHER (Protein ANalysis THrough Evolutionary Relationships) Classification System (16) and SIFT (Sorting Intolerant From Tolerant) (17), both of which (Figure S1



E

Species	Match	Gene	aa	Alignment
Human		ENST00000311322	154	E E E F N Y P L D N V H L L G Y S L G A H A A G
mutated	not conserved		154	E E E F N Y P L D N V R L L G Y S L G A H A A
Mmusculus	all identical	ENSMUSG00000015568	154	E E E F N Y P L D N V H L L G Y S L G A H A A
Ggallus	all identical	ENSGALG00000015425	126	V H L L G Y S L G A H A A
Trubripes	all identical	ENSTRUG00000002552	182	L P W E R I H L L G Y S L G A H V A
Drerio	all identical	ENSDARG00000091659	155	L D Y P L E K V H L L G Y S L G A H V A
Xtropicalis	all identical	ENSXETG00000004467	162	D D T I Q Y P I D N I H I L G Y S L G A H A A

Species	Match	Gene	aa	Alignment
Human		ENST00000311322	263	I A E R G L G D V D Q L V K C S H E R S I H L F
mutated	not conserved		263	I A E R G L G D V D Q Q V K C S H E R S I H L
Mmusculus	all identical	ENSMUSG00000015568	263	I A E R G L G D V D Q L V K C S H E R S I H L
Ggallus	all identical	ENSGALG00000015425	235	I A E K G F S D V D Q L V K C S H E R S I
Trubripes	all identical	ENSTRUG00000002552	291	I A AEGI K G L H N M D Q L V
Drerio	all conserved	ENSDARG00000091659	264	I A T C G I Y N M D Q I V K
Xtropicalis	all identical	ENSXETG00000004467	271	I A E K G F G D V D Q L V K C S H E R S I H L

Figure 2 Characterization of an Asian family of familial chylomicronemia syndrome caused by compound heterozygous variants in *LPL*. (A) The triglyceride levels in the patient. (B) Pedigree presentation of the studied family. The *LPL* p.H154R variant was colored in black while the *LPL* p.L263Q variant was colored in grey. Arrow indicated the patient. The triglyceride levels were labelled below the corresponding individuals. TG: triglyceride. (C) Sequencing analysis of the *LPL* gene showing the p.H154R variant in patient and father, and the p.L263Q variant in patient and mother. Amino acid abbreviations: V, valine; H, histidine; R, arginine; L, leucine; Q, glutamine. Nucleotide abbreviations: G, guanine; T, thymine; C, cytosine; A, adenine. (D) Visualization of the p.H154R and p.L263Q variants in the lipoprotein lipase protein structure using pymol. The residues were colored according to the following color scheme: acidic residue: red; basic residue: marine; nonpolar: orange; polar: green; cysteine: yellow. In the mutant site, both the original and mutant residues were shown. (E) Homologous protein alignment using MutationTaster2 showing the conservation of His-154 and Leu-263 residues among different species. Seq, sequencing; LPL, lipoprotein lipase.

Table 1 Laboratory testing results of the patient

Tests	Values	Reference ranges
Triglyceride	22.5 mmol/L	0–1.7 mmol/L
Total cholesterol	5.44 mmol/L	0–5.2 mmol/L
HDL-C	0.44 mmol/L	0.9–1.81 mmol/L
LDL-C	1.53 mmol/L	2.07–3.36 mmol/L
Apolipoprotein A-I	0.9 g/L	1–1.76 g/L
Apolipoprotein B	0.83 g/L	0.6–1.14 g/L
AST	35 IU/L	10–67 IU/L
γ-GT	8 IU/L	7–32 IU/L
ALT	42 IU/L	5–35 IU/L
APTT (repeated)	38.6 s	28–40 s
PT (repeated)	14.1 s	11–14.5 s
TT (repeated)	13.9 s	14–21s
Fibrinogen	1.96 g/L	2–5 g/L

HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; AST, aspartate aminotransferase; γ-GT, gamma-glutamyl transferase; ALT, alanine aminotransferase; APTT, activated partial thromboplastin time; PT, prothrombin time; TT, thrombin time.

and Figure S2, respectively), predicted that the two variants had high probability to be damaging.

In vitro experiments were carried out to validate the pathogenicity of these two *LPL* variants. First, the human *LPL* gene was cloned into pcDNA3.1 vector for expression of wild-type (WT) lipoprotein lipase. Site-directed mutagenesis was performed to construct expression vectors with *LPL* p.H154R or p.L263Q variant. Human embryonic kidney 293 cells were transfected with empty vector, WT, *LPL* p.H154R or *LPL* p.L263Q plasmids using lipofectamine 3000 (Invitrogen). The expression levels of WT and mutant proteins were quantified by ELISA (Cloud-Clone Corp). The results showed that the p.H154R variant caused significantly reduced expression of lipoprotein lipase, while the protein expression levels were comparable between WT and *LPL* p.L263Q (*Figure 3A*). To determine how the two mutants affected protein function, we quantified the lipolytic activity of WT and mutant proteins using an LPL activity assay kit (Sigma-Aldrich). The results showed that both mutants displayed significantly reduced catalytic activity compared to the WT vector (p.L263Q < p.H154R < WT) (*Figure 3B*).

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee(s) and with the Helsinki Declaration (as revised in 2013). Written informed consent was obtained from the patient's guardian for publication of this case report. A copy of the written consent is available for review by the editorial office of this journal.

Discussion

Fredrickson type 1 hyperlipoproteinemia, also known as familial chylomicronemia syndrome, has an estimated prevalence of 1 per million, which is one of the lowest among all hyperlipoproteinemia subtypes (18). In this family, the patient showed early presentation of severe hypertriglyceridemia (22.31 mmol/L) and splenomegaly. Three other family members who had heterozygous variant also presented with increased TG levels, hinting a familial inheritance pattern. Whole exome sequencing revealed the patient had compound heterozygous variants in the *LPL* gene (p.H154R and p.L263Q). *LPL* p.H154R was inherited from her father whereas *LPL* p.L263Q was inherited from her mother. The clinical and genetic findings corroborated the diagnosis of FCS in the patient. Acute pancreatitis is a characteristic of FCS. According to the APPROACH study, the percentage of FCS patients with the first pancreatitis event before 5 years old was around 15% (19). In addition, diet variation is an important factor in the initial timing of

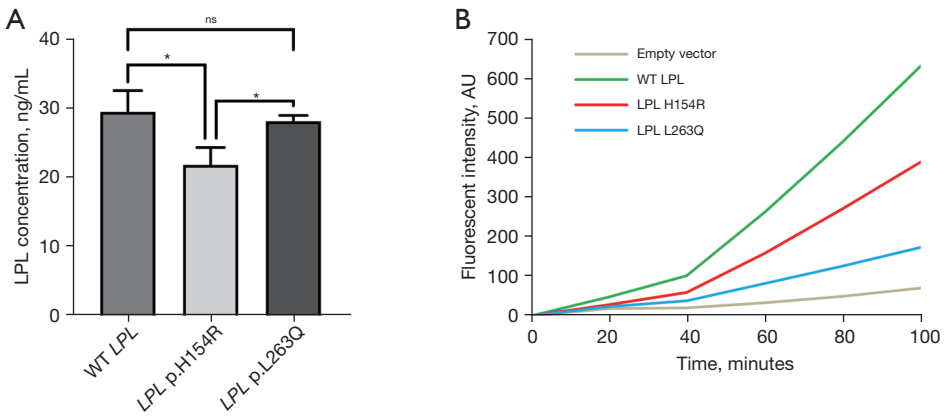


Figure 3 Functional validation of the *LPL* pathogenic variants *in vitro*. (A) Quantification of the expression levels of wild-type and mutant lipoprotein lipase proteins using ELISA. The *LPL* p.H154R variant resulted in reduced level of lipoprotein lipase whereas the *LPL* p.L263Q variant did not alter lipoprotein lipase level when compared to wild type (WT) control. (B) Comparison of the lipolytic activity of wild-type and mutant lipoprotein lipase proteins. One-way ANOVA, *, P<0.05; ns, non-significant. LPL, lipoprotein lipase; AU, arbitrary unit; ANOVA, analysis of variance.

acute pancreatitis. This may explain why our patient did not have pancreatitis when diagnosed.

During the diagnosis of this patient, it is worth noting that the patient was referred to our center due to abnormal coagulation profile. Optical analyzers are widely used in coagulation profiling. Lipemia, especially the increased chylomicron level, is an important factor during testing as it can increase plasma turbidity (13). Therefore, caution should be taken when interpretating the coagulation profile of FCS patients due to increased plasma turbidity caused by the high chylomicron levels. If there are abnormal coagulation profiles measured by turbidity-based assay, the possibility of chylomicronemia should be considered and a repeat coagulation assay should be performed using other methods that are not based on turbidity, such as magnetic bead method.

Although some family members had hypertriglyceridemia for a long time, the underlying genetic defects were only discovered due to the workup for a compound heterozygous patient with significantly abnormal findings. Interestingly, both *LPL* pathogenic variants (p.H154R and p.L263Q) in this family have not been functionally characterized before. Further *in vitro* testing supported the pathogenicity of these variants as we found that the *LPL* p.H154R variant reduced the expression of lipoprotein lipase and decreased its lipolytic activity, while the *LPL* p.L263Q variant mainly impaired its lipolytic activity. Our analyses do not explain the mechanism of reduced LPL level in the p.H154R mutant cell line. However, we might speculate that this variant might alter the structure of LPL and led to protein instability. To validate this hypothesis, further studies such as hydrogen-deuterium exchange (HDX) coupled with mass spectrometry (HDXMS) could be performed. One limitation of our study is that LPL activity and mass level were not measured using post-heparin plasma sample of the patient, which had been performed by other groups to validate FCS (20). The prevalence of loss-of-function (LOF) *LPL* variant carrier is varying among different studies, which is affected by the study populations and *LPL* variant types (21-23). Due to founder effects, some populations have higher carrier frequencies of LOF *LPL* variants than others. For example, the carrier frequency of defective *LPL* alleles is as high as 1 in 40 in some regions of the French Canadian population, which has the highest prevalence of homozygous LPL deficiency around the world (24,25). Unfortunately, such carrier frequency data was not available for the Chinese population. The impact of LOF *LPL* variant can also be varying. There is great heterogeneity in lipoprotein lipase activity and plasma TG levels (ranged from normal to severe) among heterozygous carriers with LOF *LPL* variants, which could be affected by factors such as underlying polygenic risk variants of hypertriglyceridemia, hyperinsulinemia and abdominal obesity (26-29). The contributing role of triglycerides and triglyceride-rich lipoproteins in atherosclerotic cardiovascular disease is supported by mutational analyses, genome-wide association studies, and Mendelian randomization studies (30,31). Studies are

ongoing to identify effective measures to lower triglyceride-rich lipoproteins and remnants to reduce the risk of atherosclerosis (30,31).

Regarding treatment, strict low-fat diet, with fat kept below 15% of daily energy take, helped to reduce the patient's TG levels. However, the patient's response to fenofibrate was minimal and the therapy was discontinued. For the treatment of FCS, a therapeutic lifestyle change including restricted fat intake should be initiated first. Pharmacologically, current lipid-lowering therapies include statins, fibrates, niacin, and omega-3 fatty acids (32). Unfortunately, these drugs are not quite effective in FCS patients, who may still have high TG levels and recurrent acute pancreatitis. Apolipoprotein C-III (apoC-III) and angiopoietin-like 3 (ANGPTL3) are physiological lipoprotein lipase activity inhibitors. Therefore, antisense oligonucleotide targeting apoC-III and monoclonal antibody against ANGPTL3 are developed and being tested in clinical trials to see whether they can effectively preserve LPL-mediated lipolysis of TG (33,34). Alipogene tiparvovec (Glybera) is a novel gene therapy which uses the adeno-associated virus type I vector encoding a hyper-functional version of lipoprotein lipase to reduce TG levels in FCS patients (35,36). However, the therapeutic effect of alipogene tiparvovec cannot be sustained over 6 months, and this drug was discontinued in the European market (33).

In summary, we present a family of familial chylomicronemia syndrome caused by compound heterozygous *LPL* pathogenic variants (p.H154R and p.L263Q). The *LPL* p.H154R variant reduced the expression of lipoprotein lipase and decreased its lipolytic activity, while the novel *LPL* p.L263Q variant mainly impaired its lipolytic activity.

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Footnote

Reporting Checklist: The authors have completed the CARE reporting checklist. Available at https://tp.amegroups.com/article/view/10.21037/tp-22-15/rc

Conflicts of Interest: Both authors have completed the ICMJE uniform disclosure form (available at https://tp.amegroups.com/article/view/10.21037/tp-22-15/coif). The authors report that this study was funded by Jiangsu Provincial Special Program of Medical Science (BL2012005); Jiangsu Province's Key Medical Center (ZX201102); The Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD). The authors have no other conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee(s) and with the Helsinki Declaration (as revised in 2013). Written informed consent was obtained from the patient's guardian for publication of this case report. A copy of the written consent is available for review by the editorial office of this journal.

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c.461A>G, p.H154R

PANTHER HMM: LIPASE (PTHR11610)

substitution	preservation time	Message
H154R	1037	probably damaging

PSEP (position-specific evolutionary preservation) measures the length of time (in millions of years) a position in current protein has been preserved by tracing back to its reconstructed direct ancestors. The longer a position has been preserved, the more likely that it will have a deleterious effect. The thresholds we chose were: "probably damaging" (time > 450my, corresponding to a false positive rate of ~0.2 as tested on HumVar), "possibly damaging" (450my > time > 200my, corresponding to a false positive rate of ~0.4) and "probably benign" (time < 200my).

c.788T>A, p.L263Q

PANTHER HMM: LIPASE (PTHR11610)

substitution	preservation time	Message
L263Q	455	probably damaging

PSEP (position-specific evolutionary preservation) measures the length of time (in millions of years) a position in current protein has been preserved by tracing back to its reconstructed direct ancestors. The longer a position has been preserved, the more likely that it will have a deleterious effect. The thresholds we chose were: "probably damaging" (time > 450my, corresponding to a false positive rate of ~0.2 as tested on HumVar), "possibly damaging" (450my > time > 200my, corresponding to a false positive rate of ~0.4) and "probably benign" (time < 200my).

Figure S1 The pathogenicity prediction of patient's *LPL* mutants using PANTHER (Protein ANalysis THrough Evolutionary Relationships) classification system. The two mutants (c.461A>G; p.His154Arg and c.788T>A; p.Leu263Gln) were analyzed using PANTHER and their PSEP (position-specific evolutionary preservation) was calculated. According to the analysis, both mutants were predicted to be 'probably damaging'.

c.461A>G, p.H154R

Input	Protein_id	Codon change	Mutation	Type	Score	Prediction (cutoff=-2.5)	#Seq	#Cluster	Score	Prediction (cutoff=0.05)	Median_info	#SEQ
8,19810852, A,G	ENSP00000309757	GTC C[A/G]T CTC	H154R	Single AA Change	-7.65	Deleterious	243	30	0.001	Damaging	2.82	189

c.788T>A, p.L263Q

Input	Protein_id	Codon change	Mutation	Type	Score	Prediction (cutoff=-2.5)	#Seq	#Cluster	Score	Prediction (cutoff=0.05)	Median_info	#Seq
8,19813364,T ,A	ENSP00000309757	CAG C[T/A]A GTG	L263Q	Single AA Change	-2.25	Neutral	243	30	0.025	Damaging	2.85	185

Figure S2 The pathogenicity prediction of patient's *LPL* mutants using SIFT (Sorting Intolerant From Tolerant). The two mutants (c.461A>G; p.His154Arg and c.788T>A; p.Leu263Gln) were analyzed using SIFT and both were predicted to be 'damaging'.

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