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Original Article

# Characterization of two novel pathogenic variants at compound heterozygous status in lipase maturation factor 1 gene causing severe hypertriglyceridemia

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**KEYWORDS:**
Functional assay;
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LPL activity;
Pathogenicity evaluation;
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**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, apolipoprotein C2, glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1, 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.

**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.

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**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* were functionally demonstrated as causative of severe hypertriglyceridemia.
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## 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 lipemia 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. 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 LPL.[3]

LMF1 is required for LPL folding and/or dimerization, while GPIHBP1 is an anchor protein that retains LPL at the endothelial surface.[4–6] Only few families with severe hypertriglyceridemia due to *LMF1* causative variants have been described so far.[7–9]

Recent guidelines suggest to carefully evaluate evidence about genetic variants to claim their pathogenicity.[10] 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, 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 high density lipoprotein (HDL)-cholesterol by a homogeneous enzymatic colorimetric assay. When possible, the Friedewald formula was used to calculate low density lipoprotein (LDL)-cholesterol levels.

LPL mass was measured by enzyme-linked immunosorbent assay using colorimetric detection at the Unilabs laboratory (Unilabs, Denmark). Lipase activity in postheparin 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 polymerase chain reaction 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 described.[13] Large rearrangements in the *LPL* gene were investigated by Multiplex Ligation-dependent Probe Amplification (MLPA–MRC-Holland, The Netherlands), as previously reported.[14] Variant nomenclature follows the Human Genome Variation Society recommendations (http://varnomen.hgvs.org/). Variants were checked against the Human Gene

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