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A novel frameshift mutation in the lipoprotein lipase gene is rescued by alternative messenger RNA splicing

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BACKGROUND: Type I hyperlipoproteinemia, manifesting as chylomicronemia and severe hypertriglyceridemia, is a rare autosomal recessive disorder usually caused by mutations in the lipoprotein lipase gene (*LPL*).

OBJECTIVE: We sought to determine whether mutations in *LPL* could explain the clinical indications of a patient presenting with pancreatitis and hypertriglyceridemia.

METHODS: Coding regions of *LPL* were amplified by polymerase chain reaction and analyzed by nucleotide sequencing. The *LPL* messenger RNA transcript was also analyzed to investigate whether alternative splicing was occurring.

RESULTS: The patient was homozygous for the mutation c.767_768insTAAATATT in exon 5 of the *LPL* gene. This mutation is predicted to result in either a truncated nonfunctional LPL, or alternatively a new 5′ donor splice site may be used, resulting in a full-length LPL with an in-frame deletion of 3 amino acids. Analysis of messenger RNA from the patient showed that the new splice site is used *in vivo*.

CONCLUSION: Homozygosity for a mutation in the *LPL* gene was consistent with the clinical findings. Use of the new splice site created by the insertion mutation rescues an otherwise damaging frameshift mutation, resulting in expression of an almost full-length LPL that is predicted to be partially functional. The patient therefore has a less severe form of type I hyperlipoproteinemia than would be expected if she lacked any functional LPL.
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Introduction

Type I hyperlipoproteinemia, also known as familial lipoprotein lipase (LPL) deficiency, is a rare autosomal recessive disorder, which results from the complete or partial loss of function of both copies of the *LPL* gene. The LPL catalyzes the hydrolysis of triglycerides (TGs) in TG-rich lipoproteins such as chylomicrons and very low–density lipoprotein, and therefore its loss of function results in chylomicronemia and severe hypertriglyceridaemia.[1,2]

The *LPL* gene is encoded by 10 exons spanning 30 kb on chromosome 8. Because LPL deficiency is recessive, patients are either homozygous for a pathogenic mutation or compound heterozygous for 2 different mutations. There have been >100 mutations described in the *LPL* gene, which have been implicated in hyperlipoproteinemia. In addition, because LPL requires activation by apoC-II,

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mutations in *APOC2*, as well as *APOA5*, *GPIHBP1*, and *LMF1*, have been found to affect LPL activity, although such instances are more rare than mutations in *LPL*.[3]

From a clinical perspective, it is often difficult to predict whether an identified *LPL* mutation underlies a patient's condition and how severely a mutation might affect LPL function. Recently, a systematic classification of the pathogenicity of previously reported *LPL* mutations was published, which helps guide clinicians in interpreting mutations detected in their patients.[4] However, for novel mutations not previously reported, careful analysis of the molecular pathology of the altered *LPL* gene is necessary to help inform a predicted phenotype.

Here, we report the finding of a novel insertion mutation in *LPL*, which was predicted to completely abolish lipase activity, but further investigation suggested that its actual effect was to alter messenger RNA splicing, resulting in a partially functional lipase, consistent with the patient's clinical indications.

Materials and methods

Subject

The patient was a 25-year-old woman of Indian origin who presented with acute abdominal pain, nausea, and vomiting. She was diagnosed with uncomplicated pancreatitis based on elevated lipase (269 U/L; range 16–67) and abdominal computed tomography scan showing pancreatic edema. Her serum TG at time of admission was 25 mmol/L (<1.7), and this was considered the likely cause, in the absence of evidence of other known causes (no evidence of gallstones, she did not drink alcohol, and investigation for secondary causes including IgG4 subtyping, cystic fibrosis mutation, and schistosomiasis testing were negative).

Her fasting TGs when well varied between 4 and 7 mmol/L, with postprandial rise up to 18 mmol/L. Her total cholesterol averaged 3 to 3.8 mmol/L, high-density lipoprotein 0.3 to 0.4 mmol/L, but low-density lipoprotein could not be calculated. Her apolipoprotein B was 0.27 g/L (0.49–1.03), apoA1 0.91 (1.01–1.99), and lipoprotein(a) 19 nmol/L (0–120).

She previously had similar less severe central abdominal pain on 2 occasions in the previous 2 years, but had not sought medical attention. Her general health was excellent, and she took no alternative medications or supplements. She had never taken oral contraceptives and never been pregnant. She was slim (body mass index 21.7 kg/m^2), kept fit, had no clinical or laboratory evidence of glucose intolerance or insulin resistance, and had normal thyroid and renal function. There were no tendon or skin xanthomas or evidence of other systemic illness clinically.

She had borderline portal vein dilation (13 mm) and splenomegaly (16 cm) with no evidence of portal vein thrombosis. Her liver function tests showed isolated GGT elevation at 209 U/L (0–60). Ultrasound showed mild diffuse steatosis, but liver fibrosis scan showed no increase in liver stiffness (6.9 kPa, repeated at 4.8 kPa). Screen for other causes of chronic liver inflammation–cholestasis was negative. She had no systemic symptoms, no lymphadenopathy, normal blood count other than a mild hypochromic microcytic pattern indicative of alpha thalassemia, and normal serum protein electrophoresis. White cell enzymology showed no evidence of lysosomal enzyme defects, and acyl carnitine profile was normal.

Both parents were well, and there was no known history of consanguinity. Her father had been treated for a mixed dyslipidemia for 15 years but there was no family history of cardiovascular disease or pancreatitis in her parents or her 1 male sibling. Other details of the family, including lipid measurements, were unforthcoming.

DNA–RNA isolation and sequencing

DNA was extracted from ethylenediaminetetraacetic acid–treated peripheral blood from the proband patient using a Nucleospin Blood L kit (Machery Nagel, Düren, Germany). Saliva samples from relatives of the proband living overseas were collected using an Oragene 500 tube (DNA Genotek Inc, Ottawa, Canada) and DNA was extracted according to the manufacturer's protocol. For the *LPL* transcript analysis, total RNA was extracted from ethylenediaminetetraacetic acid–treated peripheral blood using TRIzol-LS reagent (Life Technologies, Carlsbad, CA) following the manufacturer's instructions.

Coding regions plus the intron–exon boundaries of *LPL* were amplified using polymerase chain reaction (PCR) according to standard protocols using FastStart Taq polymerase (Roche, Basel, Switzerland), and the products were sequenced using Big Dye v3.1 Terminator chemistry (Applied Biosystems, Foster City, CA) and resolved on a 3130 Genetic Analyzer (Applied Biosystems). Sequencing data were analyzed using SeqScape v3 software (Applied Biosystems) and annotated using Alamut Visual v2.7 software (Interactive Biosoftware, Rouen, France). Splicing analysis used the algorithms SpliceSiteFinder-like, MaxEntScan, NNSPLICE, GeneSplicer, Human Splicing Finder, which run within the Alamut Visual software. *LPL* nucleotide and protein numbering are based on Human Genome Variation Society guidelines, which differs by 27 amino acids compared with legacy numbering used in previous literature.

For transcript analysis, complementary DNA (cDNA) was synthesized from 2 μg RNA using a SuperScript-III First Strand Synthesis System for reverse transcriptase-PCR kit (Invitrogen, Carlsbad, CA) with random hexamers according to the supplied protocol. PCR was used to amplify a product using 2 μL of the cDNA as a template and using primers located within exons 4 and 6 (LPL_exonic_4F, CATTGCAGGAAGTCTGACCA; LPL_exonic_6R, AGCCCTTTCTCAAAGGCTTC). This product was sequenced using the same primers.

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