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Clinical and functional studies of two novel variants in the *LPL* gene in subjects with severe hypertriglyceridemia

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ABSTRACT

Background: Two novel variants (p.Arg270Gly and p.Asp308Glyfs*3) in the *LPL* gene have recently been identified in subjects with hypertriglyceridemia (HTG). In this study, we investigated clinical and genetic features of their families and examined the functional significance of these two variants *in vitro*.

Methods: Clinical and genetic data were collected. Site-directed mutagenesis and transient expression in *cld* cells were performed. Lipoprotein lipase (LPL) mass and activity were measured.

Results: *In vitro* studies showed that LPL mass and activity in the media of cells transfected with the p.Arg270Gly variant were significantly reduced. In the cell lysates, however, LPL mass was preserved but LPL activity was reduced, suggesting that the LPL defect was in the secretion and activity. For the p.Asp308Glyfs*3 variant, LPL mass in the cell lysate was relatively preserved compared to that of the wild-type, while LPL mass in the media was decreased albeit not significantly. LPL activities in the cell lysate and in the media of cells transfected with this variant were significantly reduced, suggesting that the p.Asp308Glyfs*3 variant might affect the activity, and possibly, secretion of LPL.

Conclusions: These novel variants in the *LPL* gene were likely pathogenic with the defect in secretion and/or activity.

1. Introduction

Lipoprotein lipase (LPL) functions as a dimer bound to heparin sulfate proteoglycans at the surface of the capillary endothelium to hydrolyze triglyceride in lipoproteins [1,2]. When LPL is defective, accumulation of triglyceride-rich lipoproteins (*i.e.*, chylomicron and very low-density lipoprotein [VLDL]), occurs, resulting in severe hypertriglyceridemia (HTG). At present, a variety of genetic variants of the *LPL* gene that lead to LPL deficiency have been discovered [3–6].

Recently, we have identified several rare variants in the *LPL* gene among subjects with severe HTG, two of which were novel (p.Arg270Gly and p.Asp308Glyfs*3) [7]. Although sequence examination and bioinformatics studies indirectly suggested that these variants probably affected the function of LPL, definite proof requires further experiments. In this study, we describe clinical features of the probands who harbored these novel variants and performed genetic analysis on their family members. In addition, we performed additional experiments to functionally characterize these variants *in vitro*.

2. Materials and methods

2.1. Subjects and biochemical measurements

Among the cohort of 101 Thai subjects with severe HTG (triglyceride levels ≥10 mmol/L or > 886 mg/dL on at least 2 occasions), we previously identified 1 novel heterozygous missense variant, p.Arg270Gly, and 1 novel duplication variant, p.Asp308Glyfs*3, in the *LPL* gene in 3 unrelated subjects [7]. None of these variants were found in 111 normolipidemic control subjects. The families of 3 subjects were invited to participate and their clinical and genetic features were examined. Lipid levels were measured using enzymatic methods in an automated system by Roche (Laval, Quebec, Canada). All of the studied subjects gave written informed consent and the study protocol was approved by the Ethics Committee of the Faculty of Medicine, Chulalongkorn University. The study was performed in accordance to the Declaration of Helsinki for experiments involving humans.

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2.2. The cld mutant cell line

Cell culture and transfection experiments were modified from Yin et al. as previously described [8]. The *cld* mutant cell line used is a gift from Dr. M. Peterfy (University of California, Los Angeles, USA). The *cld* cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM)-10% fetal bovine serum (FBS). For the LPL assay, a total of 2×10^5 cells were seeded in a 12-well plate (Falcon™, Fisher Scientific, Waltham, MA, USA), and transfection was started 24 h after plating when cells reached ≥90% confluence.

2.3. Expression constructs and LPL target sequences

The LPL expression construct is a full-length human *LPL* cDNA subcloned into the pcDNA6/V5-His expression vector (Invitrogen, Carlsbad, CA, USA). The C-terminus of the expressed LPL protein is fused with a V5 epitope tag. We induced 2 missense variants (p.Arg270Gly and p.Phe297Leu) and a duplication variant (p.Asp308Glyfs*3) using mutagenesis primers (Supplementary Table). A secreted human placental alkaline phosphatase (SEAP) reporter gene subcloned into the pM1 expression vector (X-extremeGENE™, Roche) was used to normalize for transfection efficiency. We used an LPL/SEAP plasmid master mix throughout all experiments. The Miniprep kit (Qiagen) was used to prepare all expression vector plasmids in our studies according to the manufacturer's instructions. Quantitation of diluted plasmid solutions was performed using a NanoDrop 2000 spectrophotometer (Thermo Scientific).

2.4. Cotransfection and cell harvesting

Transfection of *cld* mutant cells was achieved using the Effectene™ transfection reagent (Qiagen) according to the manufacturer's instructions at a DNA-reagent ratio of 1:10. Each well of a 12-well plate was transfected with 0.8 μg of the *LPL* target sequence and 0.2 μg of the SEAP construct. Twenty four hours after transfection, a sample of medium was taken to measure SEAP activity, and heparin was added (a final concentration of 15 U/ml). At 48 h posttransfection, samples were removed for measurement of LPL mass and LPL activity. At the end of the experiment, cells were washed twice with phosphate-buffered saline and lysed in a detergent-containing buffer (150 mM NaCl, 20 mM Tris-HCl, pH 7.5). After sonication and centrifugation, supernatants from the lysates and media were stored at −80 °C until assayed.

2.5. Detection and quantitation

SEAP activity was measured using the SEAP Reporter Assay kit (Roche) according to the manufacturer's instructions. LPL mass was measured using the LPL ELISA kit (Cell Biolabs, Inc., San Diego, CA, USA). LPL activity was measured using the LPL activity assay kit (Sigma-Aldrich, St. Louis, MO, USA) and the result was given as nmoles/ml/h, normalized to SEAP activity.

2.6. Statistical analysis

Data are presented as mean ± SD. One-way analysis of variance with posthoc analyses was used to compare data among multiple groups. P value < 0.05 was considered statistically significant. Statistical analysis was performed using SPSS software program (version 22, Chicago, IL, USA).

3. Results

3.1. Clinical and genetic features

Previously, we have identified 2 novel variants in the *LPL* gene in 3 subjects with severe HTG (Table 1).

3.2. Patient 1 (p.Arg270Gly, heterozygous)

A 45-year-old man with a 10-year history of diabetes mellitus, hypertension and renal impairment was referred for management of HTG. The patient had no history of pancreatitis. A fasting lipid profile showed total cholesterol and triglyceride levels of 14.4 mmol/L and 30.0 mmol/L, respectively (Table 1). On examination, Patient 1 was obese with a body mass index (BMI) of 34.3 kg/m^2. His diabetes mellitus was uncontrolled with a HbA_{1c} level of 11.5% (102 mmol/mol). He was treated with the combination of fibrate, statin and an oral hypoglycemic agent. In addition for the p.Arg270Gly variant, he also had a heterozygous p.Val153Met common variant (c.457G > A: rs3135507) in the *APOA5* gene. This variant has not been shown to be associated with HTG [7,9].

A further study on his family showed that his 41-year–old younger sister also had a similar heterozygous variant (Fig. 1A). She had history of diabetes mellitus, hypertension and dyslipidemia. Her total cholesterol and triglyceride levels were 4.5 mmol/L and 2.6 mmol/L, respectively, while she was treated with a combination of statin, fibrate and fish oil. Unfortunately, we did not have the pretreatment lipid values. His mother was normolipidemic, whereas his deceased father had a history of dyslipidemia.

3.3. Patient 2 (p.Asp308Glyfs*3, homozygous)

A 20-year-old man had a long history of severe HTG since the age of 3 months. Eruptive xanthoma was noted at that time. He was started on fibrate since childhood and a lipid profile while on fibrate therapy showed total cholesterol and triglyceride levels of 2.8 mmol/L and 11.0 mmol/L, respectively (Table 1). On examination, his BMI was 21.5 kg/m^2. He also had a heterozygous c.-3A > G common variant (rs 651821) in the *APOA5* gene. This particular variant has been shown to be associated with HTG [7,10–12].

His parents were first cousins and both were heterozygous for this variant (Fig. 1B). The levels of total cholesterol and triglyceride in his father were 3.7 mmol/L and 1.7 mmol/L, respectively, whereas those in his mother were 5.2 mmol/L and 5.3 mmol/L, respectively. His father had been on fibrate therapy while his mother was not.

3.4. Patient 3 (p.Asp308Glyfs*3, heterozygous)

A 39-year-old woman was pregnant when her total cholesterol and triglyceride levels were 4.8 mmol/L and 17.5 mmol/L, respectively (Table 1). Acute pancreatitis developed at 28 weeks of gestation, which responded to medical treatment with fibrate and fish oil. Her pre-pregnancy BMI was 19.2 kg/m^2.

She was also heterozygous for both the p.Val153Met (c.457G > A: rs3135507) and the c.-3A > G (rs 651821) variants in the *APOA5* gene. Since the p.Val153Met variant has not been associated with HTG, we believe that HTG might be related to the c.-3A > G variant instead [7,9–12].

Her mother reportedly had a history of HTG with a triglyceride level of 20.3 mmol/L. Genetic analysis of her family showed that her mother and two younger brothers harbored this variant (Fig. 1C).

Collectively, examination of the family members of each proband suggested that both p.Arg270Gly and p.Asp308Glyfs*3 were associated with HTG. Mild to moderate HTG was often found in subjects with heterozygous variants, whereas severe HTG was found in subjects with homozygous variants or those with heterozygous variant and other triglyceride-raising conditions, such as uncontrolled diabetes mellitus, pregnancy or other triglyceride-raising variants (for example, c.-3A > G in the *APOA5* gene) [7,10–12].

3.5. Functional analysis of two novel variants in the LPL gene

Using 3 different bioinformatic analysis programs, both of these