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Pathogenic classification of *LPL* gene variants reported to be associated with LPL deficiency

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BACKGROUND: Lipoprotein lipase (LPL) deficiency is a serious lipid disorder of severe hypertriglyceridemia (SHTG) with chylomicronemia. A large number of variants in the *LPL* gene have been reported but their influence on LPL activity and SHTG has not been completely analyzed. Gaining insight into the deleterious effect of the mutations is clinically essential.

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METHODS: We used gene sequencing followed by in-vivo/in-vitro and in-silico tools for classification. We classified 125 rare *LPL* mutations in 33 subjects thought to have LPL deficiency and in 314 subjects selected for very SHTG.

RESULTS: Of the 33 patients thought to have LPL deficiency, only 13 were homozygous or compound heterozygous for deleterious mutations in the *LPL* gene. Among the 314 very SHTG patients, 3 were compound heterozygous for pathogenic mutants. In a third group of 51,467 subjects, from a general population, carriers of common variants, Asp9Asn and Asn291Ser, were associated with mild increase in triglyceride levels (11%–35%).

CONCLUSION: In total, 39% of patients clinically diagnosed as LPL deficient had 2 deleterious variants. Three patients selected for very SHTG had LPL deficiency. The deleterious mutations associated with LPL deficiency will assist in the diagnosis and selection of patients as candidates for the presently approved *LPL* gene therapy.

LPL is an enzyme that plays a central role in the regulation of energy stores in humans by catalyzing the hydrolysis of triglyceride (TG) in TG-rich lipoproteins such as chylomicrons and VLDL.[1] LPL deficiency is an autosomal recessive disorder caused by loss-of-function mutations in the *LPL* gene leading to chylomicronemia and consequently, very severe hypertriglyceridemia with levels typically >2000 mg/dL[2] or 17 mmol/L.[3] The *LPL* gene is located on chromosome 8p22, comprising 10 exons. To date, >100 *LPL* gene variants have been described, most of which are associated with loss of catalytic activity. A list of *LPL* variants has been continuously updated over the past 15–20 years.[1,4–6] However, up to now, no systematic analysis and classification of the deleterious nature of all reported mutations have been reported. LPL-deficient patients are either homozygotes or compound heterozygous for these variants. Even rarer mutations in other genes, such as apoCII, apoA5, GPIHBP1, or LMF1, have been found to affect LPL activity and to be associated with chylomicronemia.[7–9]

As a result of absence or severely decreased LPL activity, the lipolysis of TG-rich particles is much lower in LPL-deficient patients than in normal subjects. This leads to chylomicronemia, thought to be responsible for causing most of the clinical complications of the disease. Symptoms and signs of the condition are commonly detected in infancy with repeated episodes of abdominal pain, failure to thrive, xanthomatosis, hepatosplenomegaly, and laboratory reports of lipemic plasma but may also be detected later in life. Acute recurrent pancreatitis is a severe manifestation at all ages.[1] The prevalence of LPL deficiency is estimated to be 1 to 2 per million in the general population.[1] However, in people with very SHTG or recurrent acute pancreatitis, the prevalence may be considerably higher.[10–13] Diagnosis of this rare disease is often delayed. Given the risk for acute pancreatitis, early diagnosis and intervention are crucial for optimal care.

In the present study, we developed an efficient microarray LPL chip for the detection of 128 reported mutations in the *LPL* gene associated with triglyceride levels. A classification system for variant likelihood of pathogenicity was devised to assist clinicians in the interpretation of the genotyping results. Gene therapy (alipogene tiparvovec, Glybera) has been recently developed and approved in Europe.[14–16] Detecting deleterious LPL variants will assist in the selection of patients for gene therapy.

Materials and methods

Subjects

Two hypertriglyceridemic groups of subjects were studied. Thirty-three patients thought to have LPL deficiency were provided by physicians in the Netherlands. Three hundred and fourteen SHTG patients from centers in the Netherlands and Germany took part in the study with the LPLchip. Eligibility was defined as subjects having TG ≥ 20 mmol/L or 1770 mg/dL, recorded at least once during regular medical care. The study was reviewed and approved by the Ethics Committee of Giessen and completed in accordance with the protocol and in the spirit of the ICH Harmonized Tripartite Guidelines for Good Clinical Practice, and in accordance with the ethical principles laid down in the Declaration of Helsinki. Written informed consent was obtained from each patient before any study specific procedures were performed. Patient samples were analyzed with the LPLchip. Deoxyribonucleic acid (DNA) was extracted from saliva samples using the QIAamp DNA Blood Kit from Qiagen (Germany). Saliva samples were collected with the Oragene-DNA Self-Collection Kit (DNA Genotek Inc., Canada).

A third group of 51,476 subjects, from the Copenhagen General Population Study[17] representing the general population of Copenhagen, was selected for the study of effects of common variants in *LPL* gene on triglyceride levels.

DNA array methodology

A low-density oligonucleotide microarray was developed to identify 128 variants in the *LPL* gene previously