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Molecular and functional characterization of familial chylomicronemia syndrome

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ABSTRACT

Background and aims: Familial chylomicronemia syndrome is a rare autosomal recessive disorder leading to severe hypertriglyceridemia (HTG) due to mutations in lipoprotein lipase (LPL)-associated genes. Few data exist on the clinical features of the disorder or on comprehensive genetic approaches to uncover the causative genes and mutations.

Methods: Eight patients diagnosed with familial hyperchylomicronemia with recessive inheritance were included in this study (two males and six females; median age of onset 23.0 years; mean triglyceride level 3446 mg/dl). We evaluated their clinical features, including coronary artery disease using coronary computed tomography, and performed targeted next-generation sequencing on a panel comprising 4813 genes associated with known clinical phenotypes. After standard filtering for allele frequency <1% and *in silico* annotation prediction, we used three types of variant filtering to identify causative mutations: homozygous mutations in known familial hyperchylomicronemia-associated genes, homozygous mutations with high damaging scores in novel genes, and deleterious mutations within 37 genes known to be associated with HTG.

Results: A total of 1810 variants out of the 73,389 identified with 94.3% mean coverage (×20) were rare and nonsynonymous. Among these, our schema detected four pathogenic or likely pathogenic mutations in the *LPL* gene (p.Ala248LeufsTer4, p.Arg270Cys, p.Ala361Thr, and p.Val227Gly), including one novel mutation and a variant of uncertain significance. Patients harboring *LPL* gene mutations showed no severe atherosclerotic changes in the coronary arteries, but recurrent pancreatitis with long-term exposure to HTG was observed.

Conclusions: These results demonstrate that *LPL* gene plays a major role in extreme HTG associated with hyperchylomicronemia, although the condition may not cause severe atherosclerosis.

1. Introduction

Familial chylomicronemia syndrome is an extremely rare disorder, with an estimated frequency of 1:1,000,000, characterized by an increase in plasma triglyceride levels due to the accumulation of chylomicrons [1]. This syndrome has been shown to lead to acute pancreatitis, eruptive xanthomas, and failure to thrive [2,3]. Although it has been demonstrated that hypertriglyceridemia (HTG) can be a causal risk factor for coronary artery disease [4,5], little information exists on the clinical features of patients with extreme HTG associated with hyperchylomicronemia or on their genetic backgrounds. Mutations in genes that encode lipoprotein lipase (*LPL*), apolipoprotein CII (*APOC2*), apolipoprotein AV (*APOA5*), lipase maturing factor 1 (*LMF1*), and glycosyl-phosphatidylinositol-anchored high-density-lipoprotein-binding protein 1 (*GPIHBP1*) have been found to cause this condition [6,7]. However, it is onerous and time-consuming to check all of these genes by conventional Sanger sequencing. In addition, it is possible to miss the

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true causative variants (genes) with such a strict targeted gene strategy, motivating researchers to perform comprehensive next-generation sequencing. Accordingly, we performed comprehensive genotyping and investigated the phenotypes in detail in eight patients with hyperchylomicronemia.

2. Materials and methods

2.1. Study population

Eight patients at Kanazawa University Hospital clinically suspected of having familial hyperchylomicronemia with a form of recessive inheritance, who fulfilled the criteria of developing HTG with hyperchylomicronemia at least once between April 2003 and August 2014, were included in the present study (Fig. 1). These patients had no apparent secondary causes, such as ApoE2/E2 genotype, alcohol abuse, or prolonged uncontrolled diabetes mellitus (HbA1C > 8.5%). No patients in the study were prescribed lipid-lowering medications at the time of our evaluations.

2.2. Library preparation and targeted resequencing

DNA samples were isolated from the peripheral white blood cells of each subject using a standard DNA extraction protocol [8]. The targeted resequencing was performed using an Illumina kit: a TruSight One sequencing panel comprising 4813 genes, including genes known to cause familial chylomicronemia syndrome, following the manufacturer's instructions on a desktop sequencer, MiSeq (Illumina) [9].

2.3. Bioinformatics filtering methods

To identify the causative variants among patients with familial chylomicronemia syndrome, we applied independent basic variant filtering processes commonly used in next-generation sequencing studies [10,11]. Variants were excluded if they did not meet the following criteria: high quality based on the cutoff for coverage, strand bias, and Genotype Quality score; missense or protein truncation (nonsense, essential splice-site, or frameshift) predicted by SnpEff; and minor allele frequency (MAF) < 1% in the East Asian population. In addition, we referred to clinical and genetic in-house data using the same schema from 11 patients not showing HTG as a control and filtered out the variants that existed in the controls, i.e., variants that are relatively common in our region. In further analyses, we tried to determine the causative variants by the following steps (Fig. 2). Analysis A: homozygous or compound heterozygous variants within five established genes associated with familial chylomicronemia syndrome (*LPL*, *APOC2*, *APOA5*, *LMF1*, and *GPIHBP1*) as a recessive model. Analysis B: homozygous or compound heterozygous variants with a high damaging score according to CADD prediction software (version 1.3) [12], as a recessive model. Analysis C: deleterious variants within 37 HTG-related genes identified through a genome-wide association study (GWAS) [13] (Supplemental Table 1), as a dominant model. In analyses B and C, we filtered out the variants predicted to be benign by CADD (scaled C-score < 20), which integrates diverse genome annotations and predicts the pathogenicity of nonsynonymous variants *in silico*.

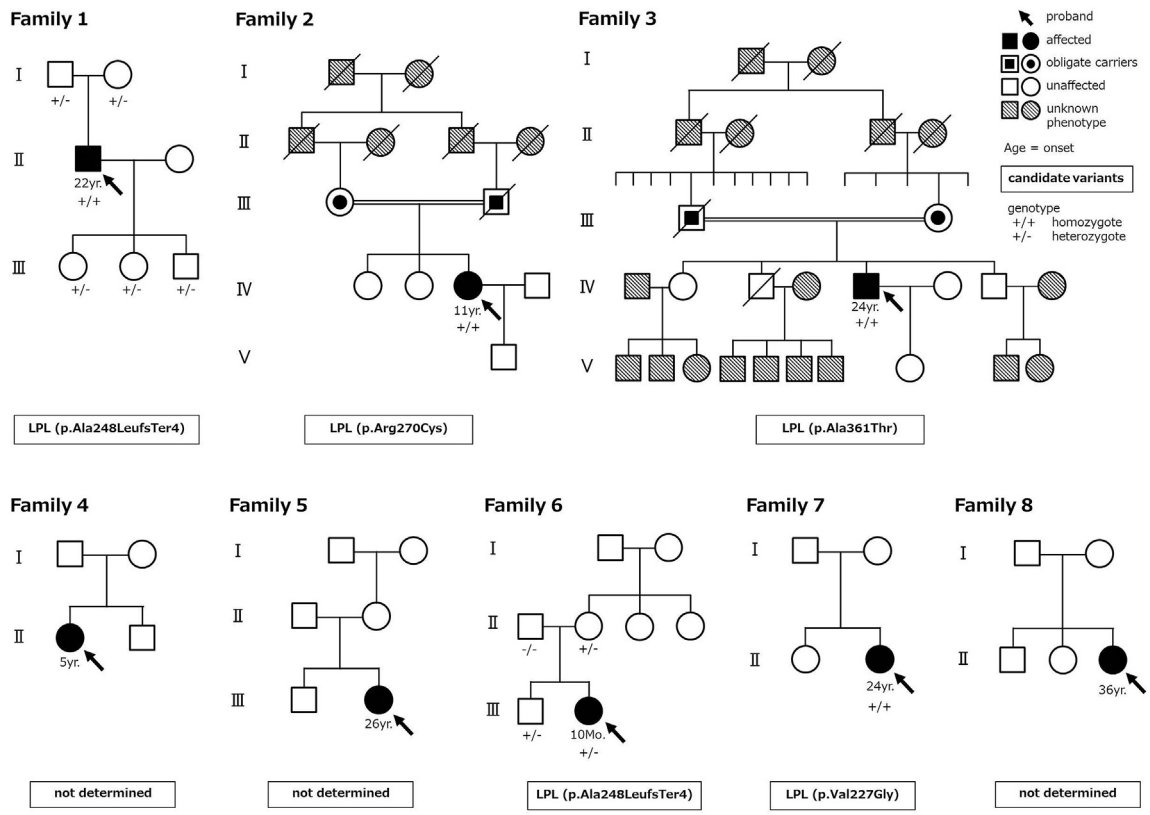

Fig. 1. Clinical and genetic profiles of families with extreme hypertriglyceridemia.
The panel-sequenced probands are indicated with arrows. Candidate variants are shown in the boxes under pedigrees. +/+, +/−, and −/− indicate homozygous variant carriers, heterozygous variant carriers, and noncarriers, respectively. LPL, lipoprotein lipase.

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