Variants of Lipid-Related Genes in Adult Japanese Patients with Severe Hypertriglyceridemia

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Aim: Hypertriglyceridemia is a type of dyslipidemia that contributes to atherosclerosis and coronary heart disease. Variants in lipoprotein lipase (*LPL*), apolipoprotein CII (*APOC2*), apolipoprotein AV (*APOA5*), glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1 (*GPIHBP1*), lipase maturation factor 1 (*LMF1*), and glucokinase regulator (*GCKR*) are responsible for hypertriglyceridemia. We investigated the molecular basis of severe hypertriglyceridemia in adult patients referred to the Clinical Laboratory at Fukuoka University Hospital.

Methods: Twenty-three adult patients with severe hypertriglyceridemia (>1,000 mg/dL, 11.29 mmol/L) were selected. The coding regions of candidate genes were sequenced by next-generation sequencing. Forty-nine genes reportedly associated with hypertriglyceridemia were analyzed.

Results: In the 23 patients, we detected 70 variants: 28 rare and 42 common ones. Among the 28 rare variants with <1% allele frequency, p.I4533L in *APOB*, p.M490I in *MLXIPL*, p.L152M in *NCAN*, and p.S264T in *TIMD4* were novel. We did not observe single gene homozygous or compound heterozygous disease-causing rare variants in any of the 23 hypertriglyceridemia cases. However, *in silico* algorithms and previous reports indicated that five rare variants, *APOA5* (p.T184S), *GCKR* (c.354 + 1G>A), *LMF1* (p.G410R), and *LRP1* (p.G813R; p.R2173Q), and seven common variants, *APOA5* (p.G185C), *APOE* (p.C130R; p.E262K/p.E263K), *GCKR* (p.V103M), *GPIHBP1* (p.C14F), *LRP1* (p.Y4054F), and *MLXIPL* (p.Q241H), can cause hypertriglyceridemia. However, all five disease-causing rare variants detected in this study were heterozygous.

Conclusions: The prevalence of disease-causing rare variants in candidate genes in severe hypertriglyceridemia patients was low. The major causes of severe hypertriglyceridemia were not single gene abnormalities, but involved multiple gene variations and environmental factors.

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Key words: Severe hypertriglyceridemia, Chylomicronemia, Variant, Mutation, Next-generation sequencing

Introduction

Numerous clinical outcome studies have shown that the levels of circulating triglyceride (TG) are an independent risk factor for coronary artery disease in humans¹⁾. Severe hypertriglyceridemia is a common lipid disorder that is associated with many comorbidities, including acute pancreatitis²⁾. Furthermore, the presence of severe hypertriglyceridemia correlates with a series of secondary factors, including extreme obesity, uncontrolled diabetes, severe liver/renal insuffi-

ciency, thyroid disease, and chemotherapy. Plasma TG is largely contained in TG-rich lipoproteins, such as very low-density lipoprotein (VLDL) and chylomicron particles. Lipoprotein lipase (LPL) is a critical enzyme in determining plasma triglyceride levels and LPL variants can result in severe hypertriglyceridemia. Moreover, apoprotein (apo)A-V, apoC-II, glycosylphosphatidylinositol-anchored high-density lipoprotein binding protein 1 (GPIHBP1), and lipase maturation factor 1 (LMF1) are also co-factors involved in the activation, transportation, or maturation

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tion of LPL. Defects in *LPL*, *APOA5*, *APOC2*, *GPIHBP1*, and *LMF1* are closely associated with chylomicronemia^{3,4}. In addition, tribbles homolog1 (TRIB1), carbohydrate response element-binding protein [ChREBP (*MLXIPL*)], and glucokinase regulator (GCKR) are all expressed in the liver and contribute to hepatic lipogenesis⁵. TRIB1 upregulates lipogenic gene expression via the indirect regulation of C/EBP α , while ChREBP directly binds to lipogenic gene promoters. When glycogen stores are full, GCKR regulates the production of glucose-6-phosphate, a substrate for lipogenesis. Hepatic lipogenesis yields triglyceride to growing nascent VLDL particles, which are subsequently secreted from the liver into the circulation.

Common variations at more than 200 genomic loci are associated at genome-wide levels of significance with one or more plasma lipid traits. However, many of the identified genes have no recognized role in lipoprotein metabolism, indicating that an incredible wealth of novel lipid biology remains to be discovered.

The majority of cases of severe hypertriglyceridemia are diagnosed in adulthood and the molecular basis of the condition has not been fully defined. There may be ethnic differences in the causative variants of hypertriglyceridemia, although this is poorly understood. In this study, we investigated the prevalence and characteristics of serum triglyceride-related gene variants in adult Japanese patients with severe hypertriglyceridemia. Forty-nine genes reported to be associated with hypertriglyceridemia, including *LPL*, *APOC2*, *GPIHBP1*, *APOA5*, and *LMF1*, were analyzed by next-generation sequencing (NGS)^{3,6-8}.

Materials and Methods

Sample Selection

This single-center study was conducted at Fukuoka University Hospital between August 2017 and October 2018. The subjects were patients who visited because of severe hypertriglyceridemia or patients in whom high triglyceride levels were detected by the hospital clinical laboratory. After an overnight fast, blood samples were collected. Twenty-three patients aged over 20 years with severe hypertriglyceridemia [TG >1,000 mg/dL (11.29 mmol/L)] were included. Average daily alcohol intake (grams of ethanol per day) was calculated from the clinical records. The subjects were divided into four subgroups according to ethanol consumption per day (nondrinkers; light drinkers: <10 g of ethanol per day; moderate drinkers: $\geq$ 10 g and <30 g of ethanol per day; heavy drinkers: $\geq$ 30 g of ethanol per day). Written informed

consent was obtained from each patient prior to DNA analysis. This study was approved by the ethics committee of Fukuoka University (#2017M009).

Whole-Exome Sequencing

Genomic DNA was extracted from blood samples using a SepaGene kit (Sekisui Medical, Tokyo, Japan), in accordance with the manufacturer's instructions. Exome enrichment was carried out with 3 μ g of genomic DNA using an Agilent SureSelect Human All Exon V6 Kit (Agilent Technologies, Santa Clara, CA), in accordance with the manufacturer's protocol. Each sample was sequenced to at least 100 $\times$ raw target depth, by the 100 bp paired-end sequencing method on the Illumina HiSeq 2000 platform (Illumina, San Diego, CA).

We checked several parameters of the fastq files, including base quality score distribution, sequence quality score distribution, average base content per read, GC distribution in the reads, PCR amplification issue, over-represented sequences, and adapter trimming. Sequence reads were trimmed where necessary and low-quality sequence reads were excluded to retain only high-quality sequences for further analysis. Adapter trimming was performed using Trimmomatic-0.36 (<http://www.usadellab.org/cms/index.php?page=trimmomatic>). Paired-end reads were aligned to the reference human genome primary assembly hg19 downloaded from the University of California Santa Cruz Genome Browser Database. Alignment was performed using BWA (version bwa-0.7.12). Paired reads that mapped to two different chromosomes were discarded from the analysis. Aligned reads were first sorted by Picard tools, and read duplicates were removed using the Picard Mark Duplicates command. After removing the duplicates, reads were realigned around the known indels provided by the Genome Analysis Toolkit (GATK) group. This was followed by a base recalibration step. After recalibration, the quality score of each base was more accurate. Known variant positions were taken into account to recalibrate the quality score. After realignment, we used GATK v3.4-46 to identify single-nucleotide variants (SNVs) and short indels. We further filtered the variants to retain good quality (depth and variant score) ones. Identified variants were annotated using Annovar (<http://annovar.openbioinformatics.org/en/latest/user-guide/download/>). To evaluate the pathogenicity of the identified nonsynonymous variants, we used SIFT (<https://sift.bii.a-star.edu.sg>), PolyPhen-2 (<http://genetics.bwh.harvard.edu/pph2/>), Mutation Taster (<http://www.mutationtaster.org>), and FATHMM (<http://fathmm.biocompute.org.uk>) programs to predict the functional consequences of the

observed amino acid substitutions. ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar/>) was also screened for variant evaluation. Splicing variants, variants pointed out by multiple in silico predictions and not excluded by ClinVar, and variants previously reported as the cause of hypertriglyceridemia were defined as disease-causing variants^{7, 9-14}. The Exome Aggregation Consortium Database (ExAC) contains allele frequency data for over 60,000 individuals, including American, Latino, East Asian, Finnish, Non-Finnish European, and South Asian (<http://exac.broadinstitute.org>) individuals. The Human Genetic Variation Database (HGVD) contains allele frequency data for 1208 Japanese individuals (<http://www.hgvd.genome.med.kyoto-u.ac.jp>). We searched for variants with an allele frequency of less than 10% in ExAC. However, for variants that had already been reported to be associated with hypertriglyceridemia, we also searched for variants with a high allele frequency^{6, 9, 15, 16}. Rare and common variants were defined as variants present at a frequency of <1% and >1% of those obtained from ExAC and HGVD, respectively. Since HGVD presents the Japanese allele frequency, the variants with <1% allele frequency in both ExAC and HGVD were defined as rare variants. The variants with >1% allele frequency in ExAC or HGVD were defined as common variants. The bam files of variants detected in NGS sequences were visually confirmed using Integrative Genomics Viewer (IGV) (<http://software.broadinstitute.org/software/igv/home>).

The following 49 genes that have been reported to be related to hypertriglyceridemia were analyzed: *AKR1C4*, *ANGPTL3*, *ANGPTL4*, *ANGPTL8*, *APOA1*, *APOA5*, *APOB*, *APOC2*, *APOC3*, *APOE*, *BLK*, *CETP*, *CILP2*, *CREB3L3*, *FADS1*, *FADS2*, *FADS3*, *FTO*, *GALNT2*, *GCKR*, *GPD1*, *GPIHBP1*, *INSR*, *IRS1*, *LDLR*, *LIPC*, *LMF1*, *LPL*, *LRPAP1*, *LRP1*, *MIR148A*, *MET*, *MLXIPL*, *MPP3*, *NAT2*, *NCAN*, *NDST1*, *NR0B2*, *PEPD*, *PIGV*, *PDXDC1*, *PINX1*, *PLTP*, *PPARA*, *SEL1L*, *TIMD4*, *TRIB1*, *VEGFA*, and *XKR6*^{6, 9, 15-25}.

Plasma Lipid and Lipoprotein Analysis

Total plasma cholesterol, TG, high-density-lipoprotein cholesterol (HDL-C), and low-density-lipoprotein cholesterol (LDL-C) levels were determined by enzymatic methods using reagents from Sekisui Medical (Tokyo, Japan). Plasma apoA-I, A-II, B, C-II, C-III, and E were measured using a turbidimetric immunoassay method from Sekisui Medical (Tokyo, Japan).

Results

Among the 23 patients with severe hypertriglyceridemia, 16 were male, 11 were obese (body mass index >25), 17 consumed alcohol (7 light, 2 moderate, and 8 heavy drinkers), 10 had diabetes, 1 had pancreatitis, and 5 had fatty liver (Table 1). Fourteen patients were taking lipid-lowering drugs. Table 2 shows plasma lipid and apolipoprotein levels at the maximum triglyceride level and the time of blood biopsy. The mean maximum triglyceride level for all 23 patients was 2107 mg/dL. As shown in Table 2, statins were given in four cases (No. 3, 6, 10, 15), but only one case (No. 23) showed LDL-C levels above 140 mg/dL. ApoB, CII, CIII, and E levels were elevated in 17 of 23 cases (No. 2, 4–13, 15–18, 21, 23).

In a whole-exome analysis of 49 genes, 70 variants were detected in 31 genes (Tables 3 and 4). Among them, there were 45 variants with <1% allele frequency in ExAC, and 28 rare variants with <1% allele frequency in both ExAC and HGVD (Japanese) (Table 4). There were 42 common variants. Of these, 17 variants had <1% ExAC allele frequency and >1% HGVD allele frequency (Japanese).

As shown in Tables 3 and 4, in 18 of the 23 patients (No. 1–5, 7, 9–12, 14, 16–19, 21–23), the following 28 rare variants were found: *ANGPTL3* (p.Y104H, No. 11), *APOA5* (p.T184S, No. 4, 14), *APOB* (p.R1388H, No. 10; p.D2065G, No. 7; p.I2950T, No.12; p.N2964S, No. 12; p.K3232E, No. 22; p.I4533L, No. 3), *CILP2* (p.A579G, No. 10; p.R1142W, No. 2), *FTO* (p.D144N, No. 24), *GALNT2* (p.G40S, No. 5), *GCKR* (c.354+1G>A, No. 21), *INSR* (p.Y1361C, No. 19), *IRS1* (p.M209T, No. 21), *LMF1* (p.M159V, No. 4; p.G410R, No. 17, 21), *LRP1* (p.G813R, No. 10; p.R2173Q, No. 1), *MET* (p.L211W, No. 3; p.M636V, No. 16), *MLXIPL* (p.M490I, No. 18), *NCAN* (p.L152M, No. 22), *NR0B2* (p.R216H, No. 3), *PDXDC1* (p.R142Q, No. 9), *TIMD4* (p.E216K, No. 14, 22; p.S264T, No. 17), and *XKR6* (p.A103S, No. 18). Among them, four variants, p.I4533L in *APOB* (No. 3), p.M490I in *MLXIPL* (No. 18), p.L152M in *NCAN* (No. 22), and p.S264T in *TIMD4* (No. 17), were novel. As shown in Table 4, we obtained four function prediction scores using PolyPhen-2, SIFT, MutationTaster, and FATHMM.

As shown in Tables 1, 3, and 4, in five patients (No. 6, 8, 13, 15, 20) only the following common variants were observed: *ANGPTL4* (p.T266M), *APOA5* (p.V153M; p.G185C), *APOB* (p.12_15del; p.R532W; p.N2785H), *APOE* (p.C130R; p.R176C), *GCKR* (p.L446P), *GPIHBP1* (p.C14F), *LIPC* (p.V95M), *LMF1* (p.P562R), and *NCAN* (p.P92S;

Table 1. Clinical characteristics and disease-causing variants of 23 patients with severe hypertriglyceridemia

No.	Age (yr)	Sex	TG Max (mg/dL)	BMI	DM	Drink-ing	Pancre-atitis	Fatty Liver	Lipid-lowering Therapy	Number of Variants		Disease-causing Variants ^s	
										Rare	Common	Rare	Common
1	22	M	1159	32.3	+	non	-	-	Bezafibrate 400 mg	1	9	<i>LRP1</i> (p.R2173Q)	<i>APOA5</i> (p.G185C), <i>MLXIPL</i> (p.Q241H)
2	41	M	1553	26.3	+	light	-	-	No	1	7		<i>APOE</i> (p.C130R)
3	54	M	2133	24.5	-	moder-ate	-	-	EPA 1,800 mg, Rosuvastatin 5 mg	3	11		<i>APOA5</i> (p.G185C), <i>GCKR</i> (p.V103M)
4	47	M	1077	36.7	-	heavy	-	+	No	2	11	<i>APOA5</i> (p.T184S)	<i>APOE</i> (p.C130R)
5	53	F	1130	19.0	-	heavy	-	-	Fenofibrate 53.3 mg	1	6		<i>GPIHBP1</i> (p.C14F*)
6	38	M	1503	31.5	+	heavy	-	-	EPA/DHA 2.0 g, Rosuvastatin 2.5 mg	0	6		
7	20	F	1555	26.2	+	non	-	+	No	1	8		<i>APOE</i> (p.E262K/p.E263K)
8	44	F	2675	16.7	-	light	-	+	Fenofibrate 53.3 mg	0	6		<i>MLXIPL</i> (p.Q241H)
9	45	M	1955	23.7	-	light	-	-	Fenofibrate 160 mg	1	11		<i>APOA5</i> (p.G185C), <i>GPIHBP1</i> (p.C14F), <i>APOE</i> (p.C130R)
10	46	M	1543	31.1	-	heavy	-	+	Pitavastatin 1 mg	3	8	<i>LRP1</i> (p.G813R)	<i>GPIHBP1</i> (p.C14F)
11	56	F	1879	23.6	+	heavy	-	+	No	1	6		<i>LRP1</i> (p.Y4054F), <i>MLXIPL</i> (p.Q241H), <i>APOE</i> (p.C130R)
12	57	F	2437	27.7	+	non	-	-	No	2	7		<i>GPIHBP1</i> (p.C14F)
13	49	M	1174	25.9	-	light	-	-	EPA 1800 mg	0	8		<i>APOA5</i> (p.G185C), <i>GPIHBP1</i> (p.C14F)
14	58	M	1208	24.1	+	non	-	-	EPA 1800 mg	2	6	<i>APOA5</i> (p.T184S)	<i>GPIHBP1</i> (p.C14F)
15	58	M	2135	22.6	+	heavy	-	-	Rosuvastatin 2.5 mg	0	7		<i>GPIHBP1</i> (p.C14F)
16	55	F	3307	19.1	-	non	-	-	Fenofibrate 80 mg	1	12		<i>APOA5</i> (p.G185C*), <i>GPIHBP1</i> (p.C14F), <i>LRP1</i> (p.Y4054F), <i>APOE</i> (p.C130R*)
17	48	M	1083	25.2	-	light	-	-	No	2	4	<i>LMF1</i> (p.G410R)	<i>GCKR</i> (p.V103M), <i>GPIHBP1</i> (p.C14F)
18	43	M	2545	29.0	+	light	-	-	Fenofibrate 53.3 mg	2	11		<i>GPIHBP1</i> (p.C14F), <i>APOE</i> (p.C130R)
19	35	F	2517	20.1	-	light	-	-	EPA/DHA 4.0 g, Fenofibrate 80 mg	1	9		<i>GPIHBP1</i> (p.C14F)
20	23	M	2767	23.3	-	heavy	+	-	No	0	8		<i>MLXIPL</i> (p.Q241H), <i>APOE</i> (p.C130R)
21	39	M	6089	35.7	-	non	-	-	No	3	8	<i>GCKR</i> (c.354+1G>A), <i>LMF1</i> (p.G410R)	<i>APOE</i> (p.C130R)
22	23	M	2459	24.6	-	heavy	-	-	No	3	7		<i>GPIHBP1</i> (p.C14F*), <i>MLXIPL</i> (p.Q241H)

(Cont. Table 1)

No.	Age (yr)	Sex	TG Max (mg/dL)	BMI	DM	Drink-ing	Pancre-atitis	Fatty Liver	Lipid-lowering Therapy	Number of Variants		Disease-causing Variants [§]	
										Rare	Common	Rare	Common
23	43	M	2349	23.6	+	moderate	-	-	EPA/DHA 4.0 g, Bezafibrate 400 mg	1	10		<i>APOA5</i> (p.G185C), <i>GPIHBP1</i> (p.C14F), <i>APOE</i> (p.C130R)

yr, years old; M, male; F, female; TG Max, maximum triglyceride value

BMI, body mass index; DM, diabetes mellitus

EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid

non, nondrinkers; light, light drinkers: <10 g of ethanol per day; moderate, moderate drinkers: ≥10 g and <30 g of ethanol per day; heavy, heavy drinkers: ≥30 g of ethanol per day

Rare, rare variants with <1% allele frequency in both ExAC and HGVD; Common, common variants with >1% allele frequency in ExAC or HGVD; *, homozygote

[§], Disease-causing variants were defined as splicing variants, variants that were identified by multiple in silico predictions and not excluded by ClinVar, and variants that were previously reported as a cause of hypertriglyceridemia.

APO, APOLIPOPROTEIN; GCKR, GLUCOKINASE REGULATORY PROTEIN; GPIHBP1, GLYCOSYLPHOSPHATIDYLINOSITOL-ANCHORED HIGH-DENSITY LIPOPROTEIN-BINDING PROTEIN 1; LMF1, LIPASE MATURATION FACTOR 1; LRP1, LOW-DENSITY LIPOPROTEIN RECEPTOR-RELATED PROTEIN 1; MLXIPL, MLX-INTERACTING PROTEIN-LIKE

p.S838N).

No nonsynonymous variants were found in 18 genes: *APOC2*, *APOC3*, *BLK*, *FADS1*, *FADS2*, *FADS3*, *GPD1*, *LDLR*, *LRPAP1*, *MIR148A*, *MPP3*, *NDST1*, *PEPD*, *PIGV*, *PLTP*, *SEL1L*, *TRIB1*, and *VEGFA*.

We detected five rare variants [*APOA5* (p.T184S), *GCKR* (c.354+1G>A), *LMF1* (p.G410R), *LRP1* (p.R2173Q; p.G813)] and seven common variants [*APOA5* (p.G185C), *APOE* (p.C130R; p.E262K/p.E263K), *GCKR* (p.V103M), *GPIHBP1* (p.C14F), *LRP1* (p.Y4054F), *MLXIPL* (p.Q241H)] that could be disease-causing (Tables 1, 3, and 4). However, we did not observe cases of hypertriglyceridemia caused by single gene homozygosity or compound heterozygosity of disease-causing rare variants.

Discussion

We searched for variants in lipid-related genes in severe adult hypertriglyceridemia patients. Severe hypertriglyceridemia is divided into two distinct primary forms. One of these, familial chylomicronemia, is a very rare monogenic early-onset chylomicronemia, which presents in childhood or adolescence. Most of these cases involve type 1 hyperlipidemia. The other form, polygenic late-onset chylomicronemia, is caused by an accumulation of several genetic variants and can be exacerbated by secondary factors, such as poor diet, obesity, alcohol intake, and diabetes mellitus. Most of these cases involve type 5 hyperlipidemia with elevated VLDL and chylomicron particles. Many genetic studies of familial chylomicronemia have been reported,

most of which excluded cases with obesity, diabetes, and alcohol consumption and focused on single gene abnormalities^{5, 26, 27}. However, the majority of patients with severe hypertriglyceridemia encountered in the clinic have at least one of these risk factors. Therefore, in this study, we did not exclude cases with such risk factors and analyzed a large number of lipid-related genes. In this study, plasma levels of apoB were elevated in 17 of 23 cases. The proportion of plasma apoB48 present in chylomicron particles is very low; therefore, elevated apoB levels indicate that most cases involved type 5 hyperlipidemia with elevated VLDL and chylomicron particles.

Severe hypertriglyceridemia, usually resulting from a combination of genetic and environmental factors, may increase the risk of acute pancreatitis. In pancreatitis resulting from severe hypertriglyceridemia, triglyceride levels were reported to be more than 1,500 mg/dL (16.935 mmol/L)²⁸. In this study, the maximum triglyceride level was over 1,500 mg/dL in 17 cases but pancreatitis was associated with only one case.

We analyzed *LPL*, *GPIHBP1*, *APOA5*, *APOC2*, and *LMF1*, which all cause familial chylomicronemia. We detected no variants in *APOC2*. In *LPL*, only p.S474X heterozygotes as a gain-of-function variant were observed in three cases (Table 3)^{3, 29}. A common variant of *GPIHBP1* (p.C14F) was found in 13 patients (No. 5, 9, 10, 12–19, 22, 23) and was homozygous in three of these. *In silico* algorithms indicated that this variant could be benign (Table 4). However, *GPIHBP1* (p.C14F) shows normal LPL binding activity, but the level of the mutant protein was reduced *in vitro*^{30, 31}. Therefore, p.C14F may be a cause of hyper-

Table 2. Serum lipid and apolipoprotein levels for 23 patients with severe hypertriglyceridemia

No.	Age (yr)	Sex	Values at the Maximum Triglyceride Level				Values at the Time of Blood Biopsy									
			TG	TC	LDL-C	HDL-C	TG	TC	LDL-C	HDL-C	ApoAI	ApoAII	ApoB	ApoCII	ApoCIII	ApoE
			(mg/dL)													
1	22	M	1159	NA	49	29	162	189	126	36	101	28.1	106	7.3	9.1	4.4
2	41	M	1553	NA	61	35	557	216	105	36	112	28.0	115	9.9	29.0	8.0
3	54	M	2133	401	NA	NA	112	142	53 [†]	53	160	28.2	68	5.9	9.8	4.6
4	47	M	1077	269	105	39	698	225	93	39	124	34.5	119	13.6	30.0	14.6
5	53	F	1130	224	81	46	278	224	126	50	148	37.2	113	9.7	18.8	5.3
6	38	M	1503	277	51 [†]	43	630	216	81 [†]	45	155	36.5	110	13.2	34.6	9.4
7	20	F	1555	NA	69	43	445	215	126	46	138	29.8	122	9.9	21.7	8.4
8	44	F	2675	447	40	33	2675*	447*	40*	33*	191	38.4	142	22.9	44.2	27.7
9	45	M	1955	259	NA	25	263	209	127	40	124	39.0	125	7.2	17.5	6.4
10	46	M	1543	275	92 [†]	45	1543*	275*	92* [†]	45*	146	39.4	122	14.3	32.4	10.9
11	56	F	1879	400	45	53	1879*	400*	45*	53*	201	47.1	131	21.2	68.3	28.1
12	57	F	2437	378	29	37	1826	313	32	36	138	30.6	132	28.0	81.5	20.8
13	49	M	1174	293	74	35	367	198	86	33	114	27.2	115	9.3	22.0	8.4
14	58	M	1208	NA	53	28	1208*	158.5*	53*	28*	130	21.9	88	6.2	18.7	8.7
15	58	M	2135	230	NA	45	1190	211	119 [†]	55	179	44.4	139	18.4	39.5	11.0
16	55	F	3307	377	45	26	2297	343	49	26	127	30.0	126	22.8	43.8	28.7
17	48	M	1083	309	94	39	1083*	309*	94.2*	39*	131	35.9	135	15.2	42.6	17.6
18	43	M	2545	294	57	41	2545*	294*	56.5*	41*	147	37.2	134	13.1	55.4	17.1
19	35	F	2517	297	76	17	2517*	297*	76*	17*	91	23.5	81	18.7	26.2	16.1
20	23	M	2767	645	NA	19	282	201	110	47	152	30.1	95	5.1	18.5	6.6
21	39	M	6089	677	31	29	6089*	677*	31*	29*	120	29.4	145	42.4	139.2	53.6
22	23	M	2459	216	62	41	2459*	216*	62.4*	41*	161	30.8	62	<0.3	12.8	5.4
23	43	M	2349	NA	108	39	252	307	218	44	125	36.7	164	7.0	12.6	6.4

yr, years old; M, male; F, female

NA, not available; *, same values as maximum triglyceride level; [†], statins were given in four cases

triglyceridemia. Among the 23 patients, 16 were heterozygous for *APOA5* variants. A disease-causing rare heterozygous variant, p.T184S, was found in two patients (No. 4, 14). A disease-causing common variant, p.G185C, was detected in six patients (No. 1, 3, 9, 13, 16, 23)³²⁻³⁴. Variants in *LMFI* were found in eight patients. One patient (No. 4) had two heterozygous variants, one rare (p.M159V) and one common (p.P562R). p.G410R was heterozygous in two patients (No. 17, 21) and was predicted to be disease-causing

by *in silico* analysis¹¹.

APOE variants affect triglyceride levels^{9, 10} and were observed in 10 patients (Table 3). Among disease-causing common variants, p.C130R was found in eight cases. Another disease-causing common variant, p.E262K, p.E263K, was heterozygous in one case (No. 7). *LIPC* encodes hepatic triglyceride lipase. A common variant of *LIPC*, p.V95M, was found in 14 cases including one homozygote; however, p.V95M is common in the Japanese population, as shown in Table 4,

Table 3. Genetic variants of the main candidate genes in 23 patients with severe hypertriglyceridemia

No.	Age (yr)	Sex	TG Max (mg/dL)	APOE Phenotype	ANGPTL3	ANGPTL4	APOA5	APOB	APOE	CILP2
1	22	M	1159	3/3	-	-	p.G185C ^{\$}	p.12_15del, p.N2785H	-	-
2	41	M	1553	3/4	-	-	-	-	p.C130R ^{\$}	p.R1142W [#]
3	54	M	2133	2/3	-	p.T266M	p.G185C ^{\$}	p.12_15del*, p.I4533L [#]	p.R176C	-
4	47	M	1077	3/4	-	-	p.T184S [#]	p.R4270T	p.C130R ^{\$}	-
5	53	F	1130	3/3	-	p.T266M	p.V153M	-	-	-
6	38	M	1503	3/3	-	-	p.V153M	p.N2785H	-	-
7	20	F	1555	3/7	-	p.V123M	-	p.12_15del*, p.D2065G [#]	p.E262K/ p.E263K ^{\$}	-
8	44	F	2675	3/3	-	-	p.V153M	-	-	-
9	45	M	1955	3/4	-	-	p.V153M, p.G185C ^{\$}	-	p.C130R ^{\$}	-
10	46	M	1543	3/3	-	-	-	p.D550H, p.R1388H [#]	-	p.A579G [#]
11	56	F	1879	3/4	p.Y104H [#]	p.T266M	-	-	p.C130R ^{\$}	-
12	57	F	2437	3/3	-	p.T266M	p.V153M	p.I2950T [#] , p.N2964S [#]	-	-
13	49	M	1174	3/3	-	p.T266M	p.G185C ^{\$}	-	-	-
14	58	M	1208	3/3	-	-	p.T184S [#]	p.R532W, p.N2785H	-	-
15	58	M	2135	3/3	-	-	p.V153M	-	-	-
16	55	F	3307	4/4	-	p.V123M	p.G185C ^{*\$}	p.D550H, p.E3788K	p.C130R ^{*\$}	-
17	48	M	1083	3/3	-	p.T266M	-	-	-	-
18	43	M	2545	3/4	-	-	p.V153M	p.R532W	p.C130R ^{\$}	-
19	35	F	2517	3/3	-	-	-	p.R532W	-	-
20	23	M	2767	3/4	-	-	-	p.12_15del	p.C130R ^{\$}	-
21	39	M	6089	3/4	-	-	p.V153M	p.T194M	p.C130R ^{\$}	-
22	23	M	2459	3/3	-	-	p.V153M	p.T194M, p.K3232E [#]	-	-
23	43	M	2349	3/4	-	p.T266M	p.V153M, p.G185C ^{\$}	p.R532W [*]	p.C130R ^{\$}	-

(Cont Table 3)

No.	GCKR	GPIHBP1	LIPC	LMF1	LPL	LRP1	MLXIPL	TIMD4
1	-	-	-	p.P562R	p.S474X	p.R2173Q [#] \$	p.Q241H ^{\$} , p.A358V	-
2	-	-	p.V95M	-	p.S474X	-	-	-
3	p.V103M ^{\$} , p.L446P [*]	-	p.V95M	p.P562R	-	-	-	-
4	p.L446P	-	p.V95M	p.M159V [#] , p.P562R	p.S474X	-	-	-
5	-	p.C14F ^{*\$}	-	-	-	-	-	-
6	p.L446P	-	p.V95M	-	-	-	-	-
7	-	-	p.V95M	-	-	-	-	-
8	p.L446P	-	p.V95M	-	-	-	p.Q241H ^{\$} , p.A358V	-
9	p.L446P	p.C14F ^{\$}	p.V95M	p.P562R	-	-	-	-
10	p.L446P	p.C14F ^{*\$}	p.V95M	-	-	p.G813R [#] \$	-	-
11	p.L446P [*]	-	-	-	-	p.Y4054F ^{\$}	p.Q241H ^{\$} , p.A358V	-
12	-	p.C14F ^{\$}	-	p.P562R	-	-	-	-
13	p.L446P	p.C14F ^{\$}	p.V95M	p.P562R	-	-	-	-
14	-	p.C14F ^{\$}	-	-	-	-	-	p.E216K [#]
15	p.L446P	p.C14F ^{\$}	-	-	-	-	-	-
16	p.L446P	p.C14F ^{\$}	-	-	-	p.Y4054F ^{\$}	-	-
17	p.V103M ^{\$} , p.L446P [*]	p.C14F ^{\$}	-	p.G410R [#] \$	-	-	-	p.S264T [#]
18	-	p.C14F ^{\$}	p.V95M	-	-	-	p.M490I [#]	-
19	p.L446P [*]	p.C14F ^{\$}	p.V95M	-	-	-	-	-
20	p.L446P	-	p.V95M [*]	-	-	-	p.Q241H ^{\$} , p.A358V	-
21	c.354 + 1G > A [#] \$, p.L446P [*]	-	p.V95M	p.G410R [#] \$	-	-	-	-
22	p.L446P	p.C14F ^{*\$}	-	-	-	-	p.Q241H ^{\$} , p.A358V	p.E216K [#]
23	p.L446P [*]	p.C14F ^{\$}	p.V95M	-	-	-	-	-

yr, years old; M, male; F, female; TG Max, maximum triglyceride value

^{*}, homozygote; [#], rare variants with < 1% allele frequency in both ExAC and HGVD; ^{\$}, disease-causing variants (Disease-causing variants were defined as splicing variants, variants that were identified by multiple in silico predictions and not excluded by ClinVar, and variants that were previously reported as a cause of hypertriglyceridemia.)

ANGPTL, ANGIOPOIETIN-LIKE; APO, APOLIPOPROTEIN; CILP2, CARTILAGE INTERMEDIATE LAYER PROTEIN 2; GCKR, GLUCOKINASE REGULATORY PROTEIN; GPIHBP1, GLYCOSYLPHOSPHATIDYLINOSITOL-ANCHORED HIGH DENSITY LIPOPROTEIN-BINDING PROTEIN 1; LIPC, HEPATIC LIPASE; LMF1, LIPASE MATURATION FACTOR 1; LPL, LIPOPROTEIN LIPASE; LRP1, LOW DENSITY LIPOPROTEIN RECEPTOR-RELATED PROTEIN 1; MLXIPL, MLX-INTERACTING PROTEIN-LIKE; NCAN, NEUROCAN CORE PROTEIN PRECURSOR; TIMD4, T-CELL IMMUNOGLOBULIN AND MUCIN DOMAINS-CONTAINING PROTEIN 4

Table 4. Genetic variants identified in candidate genes in 23 patients with severe hypertriglyceridemia

Chr	Gene	rs	Variation	Position	Hom	Het- ero	Freq in ExAC	Freq in HGVD	SIFT	Poly- phen2	MutationTaster	FA THMM	ClinVar
10	AKR1C4	rs782696885	p.L63Q	c.188T>A	0	1	0.0002	0.011259	T	B	polymorphism	T	NA
		rs3829125	p.S145C	c.434C>G	0	5	0.1371	0.082988	T	B	polymorphism	T	NA
		rs17134592	p.L311V	c.931C>G	0	5	0.1361	0.083471	T	B	polymorphism	T	NA
1	ANGPTL3	rs768762188	p.Y104H [#]	c.310T>C	0	1	0.00000854	0.000413	T	B	polymorphism	T	NA
19	ANGPTL4	rs11544610	p.V123M	c.367G>A	0	2	0.0018	0.024502	T	P	polymorphism	T	NA
		rs1044250	p.T266M	c.797C>T	0	7	0.3042	0.130833	T	B	polymorphism	T	NA
19	ANGPTL8	rs2278426	p.R59W	c.175C>T	2	7	0.1349	0.284884	D	D	polymorphism	T	NA
11	APOA1	rs12718465	p.A61T	c.181G>A	0	2	0.0029	0.042597	T	B	polymorphism	T	Likely benign
11	APOA5	rs3135507	p.V153M	c.457G>A	0	10	0.0518	0.221257	T	B	polymorphism	T	Benign
		rs201229911	p.T184S ^{#S}	c.551C>G	0	2	0.0002	0.007476	T	P	disease-causing	T	NA
		rs2075291	p.G185C ^S	c.553G>T	1	5	0.0064	0.080645	D	D	polymorphism	T	Risk factor
2	APOB	rs17240441	p.12_15del	c.35_43del	2	2	0.083	0.223594	NA	NA	polymorphism	NA	Likely benign
		rs13306198	p.T194M	c.581C>T	0	2	0.0047	0.029752	D	D	polymorphism	T	Likely benign
		rs13306194	p.R532W	c.1594C>T	1	3	0.0114	0.118595	D	D	polymorphism	T	Likely benign
		rs145862664	p.D550H	c.1648G>C	0	2	0.0002	0.026468	D	B	polymorphism	T	Likely benign
		rs13306187	p.R1388H [#]	c.4163G>A	0	1	0.0019	0.007062	T	P	polymorphism	T	Likely benign
		rs764440678	p.D2065G [#]	c.6194A>G	0	1	0.0000165	0.000413	D	P	disease-causing	T	NA
		rs2163204	p.N2785H	c.8353A>C	0	3	0.0081	0.079339	T	B	polymorphism	T	Likely benign
		rs141591543	p.I2950T [#]	c.8849T>C	0	1	0.00004944	0.00124	D	B	polymorphism	T	NA
		rs776710122	p.N2964S [#]	c.8891A>G	0	1	0.00005772	0.003306	T	B	polymorphism	T	NA
		rs544521341	p.K3232E [#]	c.9694A>G	0	1	0.0000912	NA	D	B	polymorphism	T	Uncertain significance
		rs13306191	p.E3788K	c.11362G>A	0	1	0.0001	0.013636	T	B	polymorphism	T	Uncertain significance
		rs1801702	p.R4270T	c.12809G>C	0	1	0.0456	0.044702	T	B	polymorphism	T	Likely benign
		NA	p.I4533L [#]	c.13597A>T	0	1	NA	NA	T	B	disease-causing	T	NA
19	APOE	rs429358	p.C130R ^S	c.388T>C	1	8	0.1843	0.119256	T	B	polymorphism	T	Pathogenic
		rs7412	p.R176C	c.526C>T	0	1	0.0718	0.053229	D	D	polymorphism	T	Pathogenic
		rs140808909/ rs190853081	p.E262K/ p.E263K ^S	c.784G>A/ c.787G>A	0	1	0.0004	0.01062	T	D	disease-causing	T	Pathogenic
16	CETP	rs2303790	p.D459G	c.1376A>G	0	3	0.0024	0.033058	D	P	disease-causing	T	Likely benign
19	CILP2	rs199736818	p.A579G [#]	c.1736C>G	0	1	0.00001663	0.003309	T	B	polymorphism	T	NA
		rs577556072	p.R1142W [#]	c.3424C>T	0	1	0.00004508	0.001277	D	D	polymorphism	T	NA
19	CREB3L3	rs77002741	p.G104R	c.310G>A	0	3	0.004	0.039289	T	D	polymorphism	D	NA
16	FTO	rs79206939	p.A134T	c.400G>A	0	1	0.0021	0.028099	T	B	polymorphism	T	Likely benign
		rs201086068	p.D144N [#]	c.430G>A	0	1	0.0003	NA	T	P	disease-causing	T	NA
1	GALNT2	NA	p.G40S [#]	c.118G>A	0	1	NA	0.000444	T	P	polymorphism	T	NA
		rs2273970	p.V554M	c.1660G>A	1	11	0.0853	0.301902	T	P	polymorphism	T	NA
2	GCKR	rs146175795	p.V103M ^S	c.307G>A	0	2	0.0023	0.017422	D	D	polymorphism	D	Likely pathogenic
		rs2293573	splicing ^{#S}	c.354+1G>A	0	1	8.25E-06	0.002191	NA	NA	disease-causing	NA	NA
		rs1260326	p.L446P	c.1337T>C	6	10	0.6429	0.449686	T	B	polymorphism	T	association
8	GPIHBP1	rs11538389	p.C14F ^S	c.41G>T	3	10	0.1509	0.346783	T	B	polymorphism	T	NA
19	INSR	rs13306449	p.Y1361C [#]	c.4082A>G	0	1	0.00003297	0.007851	T	D	disease-causing	T	Likely benign
2	IRS1	rs1801118	p.M209T [#]	c.626T>C	0	1	0.00007421	0.004545	T	D	disease-causing	T	NA

(Cont Table 4)

Chr	Gene	rs	Variation	Position	Hom	Het- ero	Freq in ExAC	Freq in HGVD	SIFT	Poly- phen2	MutationTaster	FA THMM	ClinVar
15	LIPC	rs3731594	p.D1137N	c.3409G>A	1	3	0.0044	0.055785	T	B	disease-causing	T	NA
16	LMF1	rs6078	p.V95M	c.283G>A	1	13	0.0744	0.237062	T	B	polymorphism	D	Benign
		rs142481016	p.M159V [#]	c.475A>G	0	1	0.0006	0.001248	T	B	polymorphism	T	NA
		rs199713950	p.G410R ^{#S}	c.1228G>A	0	2	0.0009	0.009182	D	D	disease-causing	T	NA
		rs4984948	p.P562R	c.1685C>G	0	6	0.0511	0.125	T	P	polymorphism	T	NA
8	LPL	rs328	p.S474X	c.1421C>G	0	3	0.0935	0.139883	NA	NA	polymorphism	NA	Likely benign
12	LRP1	rs199752894	p.G813R ^{#S}	c.2437G>C	0	1	0.0001	0.000846	D	D	disease-causing	D	NA
		rs370414805	p.R2173Q ^{#S}	c.6518G>A	0	1	0.0001	NA	T	D	disease-causing	D	NA
		rs79435985	p.Y4054F ^S	c.12161A>T	0	2	0.002	0.017947	T	D	disease-causing	D	NA
7	MET	rs45483396	p.L211W [#]	c.632T>G	0	1	0.0003	0.001653	D	D	disease-causing	T	Likely benign
		rs33917957	p.N375S	c.1124A>G	0	3	0.0283	0.033471	T	B	disease-causing	T	Benign
		rs761183186	p.M636V [#]	c.1906A>G	0	1	0.0000083	0.000829	T	B	disease-causing	T	NA
7	MLXIPL	rs3812316	p.Q241H ^S	c.723G>C	0	5	0.1352	0.091293	T	B	polymorphism	T	NA
		rs35332062	p.A358V	c.1073C>T	0	5	0.1049	0.108021	T	B	polymorphism	T	NA
		NA	p.M490I [#]	c.1470G>A	0	1	NA	NA	T	B	polymorphism	T	NA
8	NAT2	rs1799930	p.R197Q	c.590G>A	0	5	0.2773	0.188172	T	D	polymorphism	T	Drug response
		rs1799931	p.G286E	c.857G>A	0	7	0.0553	0.085417	T	B	polymorphism	T	Drug response
19	NCAN	rs2228603	p.P92S	c.274C>T	0	1	0.0643	0.047718	T	D	disease-causing	T	NA
		NA	p.L152M [#]	c.454C>A	0	1	NA	NA	T	D	disease-causing	T	NA
		rs10426537	p.S838N	c.2513G>A	0	1	0.0145	0.056752	T	P	polymorphism	D	NA
1	NR0B2	rs200475847	p.R216H [#]	c.647G>A	0	1	0.00009061	0.00416	D	B	disease-causing	D	NA
16	PDXDC1	rs112323280	p.L401V	c.1201C>G	0	1	0.052	0.02458	T	P	disease-causing	T	NA
		rs775458530	p.R142Q [#]	c.425G>A	0	1	0.0000494	NA	T	P	disease-causing	T	NA
8	PINX1	rs1078543	p.S254C	c.760A>T	0	4	0.1341	0.120248	T	P	polymorphism	T	NA
		rs150489215	p.C265Y	c.794G>A	0	1	0.0024	0.03843	T	B	polymorphism	T	NA
22	PPARA	rs1800234	p.V227A	c.680T>C	0	1	0.0096	0.056572	T	B	disease-causing	T	NA
5	TIMD4	rs112438191	p.E216K [#]	c.646G>A	0	2	0.00009886	0.001272	T	P	polymorphism	T	NA
		NA	p.S264T [#]	c.790T>A	0	1	NA	NA	T	P	polymorphism	T	NA
8	XKR6	rs1164344680	p.A103S [#]	c.307G>T	0	1	NA	NA	T	P	polymorphism	D	NA

Chr, chromosome; rs, reference SNP ID number

Freq in ExAC, Frequency in Exome Aggregation Consortium Database (World)

Freq in HGVD, Frequency in Human Genetic Variation Database (Japanese)

Hom, number of homozygotes; Het, number of heterozygotes; NA, not available

SIFT prediction: T, Tolerated; D, Deleterious; Polyphen2, Polymorphism Phenotyping v2: B, Benign; P, Possibly Damaging; D, Probably Damaging

FATHMM, Functional Analysis through Hidden Markov Models: T, Tolerated; D, Deleterious

[#], rare variants with <1% allele frequency in both ExAC and HGVD; ^S, disease-causing variants (Disease-causing variants were defined as splicing variants, variants that were identified by multiple in silico predictions and not excluded by ClinVar, and variants that were previously reported as a cause of hypertriglyceridemia.)

AKR1C4, ALDO-KETO REDUCTASE FAMILY 1, MEMBER C4; ANGPTL, ANGIOPOIETIN-LIKE; APO, APOLIPOPROTEIN; CETP, CHOLESTERYL ESTER TRANSFER PROTEIN; CILP2, CARTILAGE INTERMEDIATE LAYER PROTEIN 2; CREB3L3, cAMP RESPONSE ELEMENT-BINDING PROTEIN 3-LIKE 3; FTO, FAT MASS- AND OBESITY-ASSOCIATED GENE; GALNT2, GalNAc TRANSFERASE 2; GCKR, GLUCOKINASE REGULATORY PROTEIN; GPIHBP1, GLYCOSYLPHOSPHATIDYLINOSITOL-ANCHORED HIGH DENSITY LIPOPROTEIN-BINDING PROTEIN 1; INSR, INSULIN RECEPTOR; IRS1, INSULIN RECEPTOR SUBSTRATE 1; LIPC, HEPATIC LIPASE; LMF1, LIPASE MATURATION FACTOR 1; LPL, LIPOPROTEIN LIPASE; LRP1, LOW DENSITY LIPOPROTEIN RECEPTOR-RELATED PROTEIN 1; MET, MET PROTOONCOGENE; MLXIPL, MLX-INTERACTING PROTEIN-LIKE; NAT2, N-ACETYLTRANSFERASE 2; NCAN, NEUROCAN CORE PROTEIN PRECURSOR; NR0B2, NUCLEAR RECEPTOR SUBFAMILY 0, GROUP B, MEMBER 2; PDXDC1, PYRIDOXAL-DEPENDENT DECARBOXYLASE DOMAIN-CONTAINING PROTEIN 1; PINX1, PIN2-INTERACTING PROTEIN 1; PPARA, PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-ALPHA; TIMD4, T-CELL IMMUNOGLOBULIN AND MUCIN DOMAINS-CONTAINING PROTEIN 4; XKR6, XK-RELATED PROTEIN 6

and is not associated with hypertriglyceridemia¹⁷.

All three angiopoietin-like protein (ANGPTL) members, ANGPTL3, ANGPTL4, and ANGPTL8, are LPL inhibitors and deficiency or overexpression of any one of them results in hypotriglyceridemia or hypertriglyceridemia, respectively¹⁸. In *ANGPTL3*, p.Y104H was heterozygous in one patient (No. 11). The *ANGPTL4* variant was detected in nine cases (No. 3, 5, 7, 11–13, 16, 17, 23). In the *ANGPTL8* gene, p.R59W was detected in nine cases. However, we could not detect disease-causing variants in the *ANGPTL3*, 4, and 8 genes³⁵.

APOB encodes apolipoprotein B, which is found in all atherogenic lipoprotein species. Various *APOB* variants were detected in 15 of the 23 cases in this study (No. 1, 3, 4, 6, 7, 10, 12, 14, 16, 18–23). A novel variant, p.I4533L, was heterozygous in one case (No. 3). p.12_15del is associated with higher levels of apoB and LDL cholesterol without affecting triglyceride levels^{36, 37}. p.N2785H has no effect on hyperlipidemia risk in the Chinese population³⁷. No disease-causing variants were detected in the apo B gene. The glucokinase regulatory protein encoded by *GCKR* is an allosteric regulator that allows rapid mobilization of glucokinase in response to glucose concentration. For *GCKR*, three variants were observed in 16 patients. It was reported that p.P446L promoted glucose uptake and mobilization, leading to increased de novo triglyceride synthesis, whereas p.L446P had the opposite effects³⁸. The heterozygous common variant p.V103M and the rare splicing donor site variant c.354+1G>A (rs2293573) were detected in two cases (No. 3, 17) and one case (No. 21), respectively. As shown in Table 4, p.V103M was predicted to be disease-causing by *in silico* algorithms (SIFT, PolyPhen-2, and FATHMM). The low-density-lipoprotein receptor-related protein 1 (LRP1) is a member of the low-density-lipoprotein receptor (LDLR) family³⁹. LRP1 was originally identified as an endocytic receptor for α 2-macroglobulin-proteinase complexes and apolipoprotein E. LRP1 is involved in the clearance of chylomicron remnants from circulation. LRP1 functions in signal transduction pathways and can interact with other cell receptors. LRP1 is also involved in insulin signaling and glucose homeostasis in several different tissues. For *LRP1*, two rare variants, p.G813R and p.R2173Q, and the common variant p.Y4054F were heterozygous in one (No. 10), one (No. 1), and two cases (No. 11, 16), respectively. *In silico* algorithms (PolyPhen-2, Mutation Taster, and FATHMM) indicated that these three variants could be disease-causing (Table 4). *MLXIPL* encodes a transcription factor that regulates glucose utilization and lipogenesis in the liver. *MLXIPL* is associated with plasma triglyc-

eride levels in the Japanese population⁹. In *MLXIPL*, a novel mutant, p.M490I, was heterozygous in one case. A disease-causing common variant, p.Q241H, was heterozygous in five cases (No. 1, 8, 11, 20, 22)¹⁴. A common variant, p.A358V, was also reported to be associated with apoA1 values in Chinese subjects⁴⁰. The Neurocan-Cartilage intermediate layer protein 2 (*NCAN-CILP2*) region spans 300 kb on chromosome 19 and forms a tight linkage disequilibrium block. Through GWAS, many genetic variants associated with plasma lipid levels have been identified, among which the *NCAN-CILP2* region has high statistical significance and a relatively large effect size per allele on LDL cholesterol and triglyceride levels¹⁹. Two rare heterozygous variants, p.A579G (No. 10) and p.R1142W (No. 2), were found in the *CILP2* gene. For the *NCAN* gene, a novel variant p.L152M (No. 22) was heterozygous. However, no disease-causing variants were detected in the *NCAN* and *CILP2* genes. The T-cell immunoglobulin and mucin domain 4 (*TIMD4*) gene is located on chromosome 5 and is a member of the T-cell immunoglobulin domain and mucin domain gene family, which plays a critical role in regulating immune responses. *TIMD4* variants are associated with serum lipid traits, but the findings regarding their effects have been inconsistent⁴¹. For *TIMD4*, a novel variant, p.S264T, and a rare variant, p.E216K, were heterozygous in one (No. 17) and two cases (No. 14, 22), respectively. The function of XK-related protein 6 (*XKR6*) has been poorly defined²⁰. A rare variant of the *XKR6* gene, p.A103S, was heterozygous in one patient (No. 18). No disease-causing variants were detected in the *TIMD4* and *XKR6* genes.

Until recently, most genetic studies on patients with severe hypertriglyceridemia focused on *LPL* and genes that regulate *LPL*, namely, *APOA5*, *APOC2*, *GPHLBP1*, and *LMF1*; other genetic causes may thus have been neglected^{32, 42, 43}. In the current study, we performed NGS for 49 reported TG-regulating genes in 23 Japanese patients with severe hypertriglyceridemia. In this study, there were 17 variants with frequency <1% in ExAC for the global population, but >1% in HGVD for the Japanese. This appears to indicate the existence of ethnic differences in allele frequency.

Most of our cases were believed to involve type 5 hyperlipidemia. Surendran *et al.* analyzed five genes (*LPL*, *GPIHBP1*, *APOA5*, *APOC2*, and *LMF1*) in patients with severe hypertriglyceridemia³. In 29 of 43 cases (67%) of type 1 hyperlipidemia and 11 of 43 cases (26%) of type 5 hyperlipidemia, rare variants causing disease were detected. In 9 cases (21%) of type 1 hyperlipidemia and 16 cases (37%) of type 5 hyperlipidemia, common variants of *LPL* or *APOA5* were

also detected³⁾. Although the target genes and ethnicity of the subjects in the current study were different, here, five disease-causing rare variants in four genes, *APOA5* (p.T184S), *GCKR* (c.354+1G>A), *LMF1* (p.G410R), and *LRP1* (p.G813R; p.R2173Q), were detected in 6 out of 23 cases (26%). In 16 cases (70%), only 7 disease-causing common variants in six genes, *APOA5* (p.G185C), *APOE* (p.C130R; p.E262K/p.E263K), *GCKR* (p.V103M), *GPIHBP1* (p.C14F), *LRP1* (p.Y4054F), and *MLXIPL* (p.Q241H), were detected. In one case (4%), no disease-causing variants were detected (Table 1). However, we have to recognize that severe hypertriglyceridemia could involve a gene that has yet to be characterized. These cases highlight the need for physicians to consider a polygenic cause for patients with severe hypertriglyceridemia. This possibility should be evaluated using methods that screen for both rare large-effect variants and common small-effect variants. We detected several homozygotes or compound heterozygotes of disease-causing common variants, but all of the disease-causing rare variants detected in this study were heterozygotes.

This study had some limitations. First, we could not prepare a normal control group. Second, this was a single-center study and the sample size was small. Third, only exome sequencing was performed. However, to the best of our knowledge, few studies have performed detailed analysis of more than 40 genes in cases of severe hypertriglyceridemia.

Conclusion

The prevalence of rare variants in candidate genes in patients with severe hypertriglyceridemia was low. The major causes of severe hypertriglyceridemia were not single gene variants, but multiple genetic variations and environmental factors.

Conflict of Interest

The authors have no conflicts of interest to declare.

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