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TRANSLATIONAL SCIENCES

Spectrum of Mutations and Long-Term Clinical Outcomes in Genetic Chylomicronemia Syndromes

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OBJECTIVE: Familial chylomicronemia syndrome (FCS) and multifactorial chylomicronemia syndrome (MCS) are the prototypes of monogenic and polygenic conditions underlying genetically based severe hypertriglyceridemia. These conditions have been only partially investigated so that a systematic comparison of their characteristics remains incomplete. We aim to compare genetic profiles and clinical outcomes in FCS and MCS.

APPROACH AND RESULTS: Thirty-two patients with severe hypertriglyceridemia (triglyceride >1000 mg/dL despite lipid-lowering treatments with or without history of acute pancreatitis) were enrolled. Rare and common variants were screened using a panel of 18 triglyceride-raising genes, including the canonical *LPL*, *APOC2*, *APOA5*, *GP1HBP1*, and *LMF1*. Clinical information was collected retrospectively for a median period of 44 months. Across the study population, 37.5% were classified as FCS due to the presence of biallelic, rare mutations and 59.4% as MCS due to homozygosity for nonpathogenic or heterozygosity for pathogenic variants in canonical genes, as well as for rare and low frequency variants in noncanonical genes. As compared with MCS, FCS patients showed a lower age of hypertriglyceridemia onset, higher levels of on-treatment triglycerides, and 3-fold higher incidence rate of acute pancreatitis.

CONCLUSIONS: Our data indicate that the genetic architecture and natural history of FCS and MCS are different. FCS expressed the most severe clinical phenotype as determined by resistance to triglyceride-lowering medications and higher incidence of acute pancreatitis episodes. The most common genetic abnormality underlying FCS was represented by biallelic mutations in *LPL* while *APOA5* variants, in combination with high rare polygenic burden, were the most frequent genotype of MCS.

VISUAL OVERVIEW: An online visual overview is available for this article.

Key Words: familial hyperchylomicronemia ■ genetics ■ humans ■ lipoprotein lipase ■ triglycerides

Severe hypertriglyceridemia is a relatively uncommon dyslipidemia with an estimated prevalence of ≈1/600 in the general population.[1] It is usually diagnosed when fasting plasma concentration of total triglycerides exceeds 10 mmol/L (≥885 mg/dL) on multiple occasions.[2,3] The clinical relevance of severe hypertriglyceridemia is due to its association with about 2-fold higher risk of acute pancreatitis (AP)[4] with a stepwise increase of this risk over time.[5] In addition to being a potentially life-threatening medical emergency, AP may also lead to several clinical complications such as chronic pancreatitis, pancreatic insufficiency, and type 2 diabetes mellitus.[6] Although the accumulation of other TRLs (triglyceride-rich lipoproteins; eg, very-low-density lipoproteins, intermediate-density lipoproteins, and remnants) may contribute to severe hypertriglyceridemia, the hallmark lipoprotein abnormality of this phenotype is chylomicronemia, defined as the presence of circulating

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Nonstandard Abbreviations and Acronyms

AP	acute pancreatitis
FCS	familial chylomicronemia syndrome
HDL-C	high-density lipoprotein cholesterol
LDL	low-density lipoprotein
LPL	lipoprotein lipase
MCS	multifactorial chylomicronemia syndrome
MCT	medium-chain triglyceride
OR	odds ratio
TRL	triglyceride-rich lipoprotein

Highlights

- Molecularly defined familial and multifactorial chylomicronemia syndrome represent 2 distinct clinical entities as proved by a different genetic architecture and long-term outcomes.
- Familial chylomicronemia syndrome presented the most severe clinical phenotype.
- Homozygosity for *LPL* mutations was the most common genotype underlying familial chylomicronemia syndrome.
- *APOA5* variants in combination with high rare polygenic burden was the most frequent genetic determinant of multifactorial chylomicronemia syndrome.

chylomicrons in the fasting state. Indeed, it has been indicated that when fasting triglyceride raise above 1000 mg/dL invariably chylomicrons appear in the plasma.[7]

Severe hypertriglyceridemia represents a heterogeneous condition where secondary factors, namely high caloric diet, alcohol abuse, visceral obesity, insulin resistance, and genetic factors contribute to its pathogenesis.[6] Historically, the main genetic disorder causing severe hypertriglyceridemia was considered familial chylomicronemia syndrome (FCS).[7] FCS is inherited as an autosomal recessive disease, and its diagnosis is based on the detection of rare, biallelic (homozygous or compound heterozygous) mutations in *LPL* (*lipoprotein lipase*) and in other genes encoding proteins required for LPL activity, such as *APOC2*, *APOA5*, *GPIHBP1*, and *LMF1*.[2,6] Consequently, the *LPL*-mediated lipolysis of triglycerides in chylomicrons and other TRLs is severely impaired with massive accumulation of chylomicrons during fasting and postprandial state. Additional clinical symptoms associated with FCS include transient eruptive xanthomas, often appearing on the trunk and extremities, and lipemia retinalis, the milky appearance of the retinal vessels.[6] Hepatosplenomegaly resulting from triglyceride uptake by macrophages may be also present in FCS.[8] Neurological symptoms, such as irritability, memory problems, dementia, and depression, have also been documented.[3]

Typically, these patients have poor response to triglyceride-lowering medications so that their management represents a clinical challenge.[6] The cornerstone of the therapy of FCS is represented by a drastic reduction of fat intake (8%–10% of total calories), which is difficult to be maintained over time. The supplementation of diet with medium-chain fatty acids in the form of oil (medium-chain triglyceride [MCT] oil) may be another option to improve hypertriglyceridemia because these fatty acids do not promote the synthesis of chylomicrons and are directly secreted into the portal bound to albumin. Fibrates, polyunsaturated fatty acid methyl ester, and niacin are the drugs often used in combination in these patients, even if they show extremely variable therapeutic effects. Finally, plasma exchange can be used to reduce plasma triglyceride through the mechanical removal of TRLs. However, its effect is transient, and, more importantly, this procedure is not available everywhere. Overall, even if combined, these treatments rarely allow to maintain in the long term triglyceride values within the range to prevent episodes of AP.[6] More recently, it has been recognized that the severe hypertriglyceridemia phenotype may be more commonly caused by the coexistence of heterozygosity for rare variants in canonical FCS genes, as well as by the presence of rare or functional common variants in noncanonical triglyceride-raising genes. This condition has been named as multifactorial chylomicronemia syndrome (MCS)—a definition that implies that secondary factors have to interact with polygenic determinants to determine the expression of hypertriglyceridemia phenotype.[9]

Because of their considerable phenotypic overlap, monogenic and polygenic forms of severe hypertriglyceridemia are difficult to be distinguished, so that the vast majority of investigations have focused their attention almost exclusively on FCS.[10] This left several unanswered questions related to the clinical and genetic characteristics of these two severe hypertriglyceridemia genotypes. In particular, it has not been clarified whether some genes are more frequently associated to FCS than MCS. In addition, there are controversies about the prevalence of monogenic forms when triglyceride levels exceed 1000 mg/dL (or 10 mmol/L) in the absence of other exacerbating factors.[11] Finally, and more importantly, it has not been well established whether FCS and MCS differ in their response to conventional triglyceride-lowering therapies or in major clinical outcomes, namely AP. As new pharmacological treatments for severe hypertriglyceridemia (eg, volanesorsen or evinacumab) are currently under evaluation,[6] the answer to these questions could be of some help in guiding the indications of these novel therapies, as well as in the clinical management of affected patients.

Therefore, aims of our study were (1) to describe the spectrum of mutations in canonical and noncanonical genes in a cohort of patients with severe hypertriglyceridemia and (2) to compare FCS and MCS in terms of clinical outcomes.

MATERIALS AND METHODS

The data that support the findings of this study are available from the corresponding author on reasonable request.

Study Patients

Thirty-eight patients with severe hypertriglyceridemia (4 of them were first-degree relatives) were identified among 4000 hyperlipemic individuals referred to the Lipid Clinic at Policlinico Umberto I, Sapienza University Hospital (Rome, Italy), as probably affected by genetic chylomicronemia syndrome based on the following criteria: (1) plasma triglyceride levels >1000 mg/dL despite at least 6 months of triglyceride-lowering therapy (fibrates, omega-3 fatty acids, MCT) with or without (2) medical history of AP. All patients were on a low-fat diet regimen.

Exclusion criteria were alcohol abuse, uncontrolled diabetes mellitus (hemoglobin A1C [HbA_{1C}] >9% or fasting plasma glucose >140 mg/dL), severe obesity (body mass index, >40 kg/m^2), use of medications known to alter lipid metabolism.[12]

Selected patients were called by phone to explain the study and to schedule a visit at our clinical center. None refused to participate. In accordance with the principles of Helsinki, patients provided written informed consent under the approval from the Institutional Ethics Committee (approval code No. 2469). After signing, patients underwent a complete clinical evaluation and blood withdrawal. Subjects' examination was performed in the morning after overnight fast. After this preliminary examination, 6 patients were excluded for poor glycemic control, suspect of alcohol abuse, or current use of medication known to affect lipids. Therefore, 32 patients (including the 4 first-degree relatives) were considered for the genetic study.

Medical records of enrolled patients were retrospectively reviewed to obtain information about baseline clinical conditions, plasma lipid changes, treatments, and clinical outcomes. The observational period was considered as the time between the last and the first visit at our Lipid Clinic. Baseline lipid measurements were considered as those determined at first visit; on-treatment values were estimated as the mean of 3 independent measurements during the observational period (not including the best or worst results). The lowest triglyceride value observed during the observational period was also taken as the best result of triglycerides achieved. The occurrence of AP was considered as the main clinical outcome. It was evaluated as following: (1) the history of AP, (2) history of AP recurrence (>1 episode), and (3) the total number of AP events experienced during life by each patient. The incidence rates of AP in each group were calculated considering the cumulative number of AP events divided by the sum of individual ages and expressed as per 1000 person-years. Nevertheless, the occurrence of coronary artery disease and stroke was also considered in the analysis of the atherosclerotic cardiovascular disease complications.

The observational period in the whole cohort ranged from 2 to 204 months (median, 44 months).

Biochemical Determinations

Blood samples were collected early in the morning after an overnight fast in EDTA-containing tubes. Plasma concentrations of lipoprotein and blood glucose were determined as described previously.[13]

Genetic Analysis

Genetic analysis was performed by targeted next-generation sequencing using a customized hypertriglyceridemia panel. This panel included 18 genes *LPL*, *APOA5*, *LMF1*, *GPIHBP1*, *APOC2*, *APOC3*, *APOE*, *ANGPTL3*, *ANGPTL4*, *ANGPTL8*, *CREB3L3*, *GALNT2*, *GCKR*, *GPD1*, *LIPC*, *LIPG*, *MLXIPL*, and *TRIB1*, which have all been reported to harbor pathogenic and susceptibility variants for elevated triglyceride.[10,14–16]

The target regions were designed by capturing all coding exons and 25 nucleotides upstream and downstream at exon-intron junctions. Illumina Design Studio (Illumina, Inc, San Diego, CA) was used to design oligo-probes and to create a custom target enrichment library of the selected genes. The final design consisted of 300 amplicons with an average size of 250 bp and a cumulative targeted region of 35.328 bp. Panel was designed to cover 100% of target with a mean depth coverage of ≈300×. Amplicon sequencing libraries were prepared from 10 ng of DNA per sample according to the TruSeq Amplicon protocol (Illumina, Inc; document No. 1000000002191 v03). The pooled libraries were paired end (2×150) and sequenced on a micro flow cell with V2 300 chemistry on a MiSeq instrument (Illumina, Inc).

Sequencing quality control and primary analysis were performed using Illumina Real Time Analysis Software.[17] After demultiplexing and generation of FASTQ files, sequence alignment to the reference genome and sequence quality filtering were performed using MiSeq Reporter v2.3 software. Variant calling was performed on the variant call format output files by evaluating the mean depth coverage and the quality score (Q score; the estimated probability of the base call being wrong). Specifically, we filtered only nonsynonymous exonic and splice-site variants according to the following pipeline: (1) mean depth coverage of at least 50×; (2) a Q score≥30 (an error rate in base calling of 1 in 1000) and genotype quality=99; (3) at least 40% of the reads supporting alternative allele (variant frequency) and, therefore, balanced allelic depth. All variants not passing the criteria reported above were considered only if validated by Sanger sequencing.[18] Generated variant call format files were then merged in a single variant call format file and subjected to annotation using wANNOVAR software.[19]

Nonsynonymous, nonsense, frameshift and splice-site variants were classified by minor allele frequency (MAF) as rare (MAF <0.01), low frequency (MAF from 0.01 to 0.05), and common (MAF >0.05),[20] after exploring public available resources in Single Nucleotide Polymorphism Database (dbSNP), Exome Aggregation Consortium (EXAC), and Genome Aggregation Database (gnomAD).[21] Their predicted functional effect was evaluated by SIFT, PolyPhen-2 HDIV, PolyPhen-2 HVAR, Mutation Taster, and Combined Annotation Dependent Depletion.[21] Variants were defined as probably damaging if reported as deleterious in at least 3 of 5 in silico prediction tools (mutation score, ≥3). In addition, variants were considered pathogenic if previously associated with FCS according to PubMed central references, Human Gene Mutation Database, and Online Mendelian Inheritance in Man annotations.[21] Whenever possible, novel variants, as well as those known but never associated with FCS, were further tested by investigating their segregation in family members using Sanger sequencing.

Patients carrying biallelic pathogenic rare variants in canonical *LPL*, *APOA5*, *LMF1*, *GPIHBP1*, and *APOC2* genes were diagnosed as FCS. All other identified rare and low-frequency

heterozygous variants were used for the MCS group classification.[2,3] Notably, the common variant p.R19W in *APOA5* (rs3135506) was used for the molecular diagnosis of MCS if present both at homozygous and heterozygous state.[22–24] The patients were defined as undetermined if not carrying rare or low-frequency variants in any of the sequenced genes.

Evaluation of Polygenic Risk

The polygenic burden was evaluated by considering rare, low-frequency, and common variants found in all screened genes. The mutations used to define FCS were excluded. First, we compared the total number of pathogenic and nonpathogenic variants in FCS and MCS groups. Second, we applied a linear weighting of 0, 1, and 2 to genotypes for all variants containing 0, 1, or 2 risk alleles,[18] thus producing an unweighted allele triglyceride score based on 18 genes ranging from 2 to 10 in our sample. In addition, stratifying for allele frequencies, we calculated the burden of rare and common triglyceride risk alleles, thus producing an unweighted rare-allele triglyceride score across 35 SNP loci (ranging from 0 to 6) and an unweighted common-allele triglyceride score across 10 SNP loci (ranging from 1 to 9). To confirm the ability of triglyceride scores in discriminating between phenotypes, we have genotyped for 35 rare and low-frequency SNPs a group of 94 age- and sex-matched individuals presenting triglyceride <150 mg/d (mean value, 86.3±17.2 mg/dL; range, 60–143). These individuals have been recruited from a population of 2069 blood donors registered at the Department of Transfusion Medicine as described previously.[13] Genotyping was performed by using the Fluidigms microfluidic array technology, and the end point genotype data were analyzed using the Fluidigm SNP Genotyping Analysis software according to the manufacturer's protocols.[25]

Statistical Analysis

Data are presented as mean (±SD) or percentage as appropriate. Differences in frequencies between FCS and MCS were tested using χ^2 statistics. The 2-sample *t* test was used to compare differences between FCS and MCS groups for quantitative traits. Logistic regression analysis methods were adopted to assess the odds ratios (ORs) conferred by triglyceride risk alleles, to evaluate AP and predictors of FCS and MCS phenotypes. The linear association analysis was used to estimate differences in unweighted allelic triglyceride score between groups and differences between AP outcomes.

A *P* value <0.05 was considered as statistically significant. All analyses have been performed using SPSS software (version 20).

RESULTS

Characteristics of Study Patients

The baseline characteristics of patients are reported in Table 1. Twenty-eight were Italians and 4 were from Asia; 65.6% of patients were men. Patients were middle aged and slightly overweight even though a broad range of body mass index was observed. The reported age of hypertriglyceridemia onset was 26.1±14.3 years. Ten patients (31.3%) had a history of diabetes mellitus and 11 (34.4%) of hypertension. A minority (12.5%) had a history of coronary artery disease. Half of the patients reported a history of AP, with a mean age of onset of 28.9±13.3 years. None of the patients had *APOE* E2/E2 genotype, while 50% had either E3/E2 (n=8) or E4/E3 (n=8) genotypes. Moreover, the *APOA5* **1/*2, *2/*3* and **3/*3* haplotypes were found in 10 patients, respectively, while APOA5 **1/*3* and **2/*2* haplotypes were found in 2 and 1 patients.[22–24] Mean baseline triglyceride levels were 2066.6±841.8 mg/dL (range, 1019.0–4480.0 mg/dL), and this was associated with increased non-HDL (high-density lipoprotein) cholesterol (HDL-C; mean, 266.1±109.5 mg/dL) and very low HDL-C levels

Table 1. Baseline Characteristics of Enrolled Patients With Severe HTG

Variables	Mean (±SD)	Range
n	32	
Age, y	42.2±13.4	18.0–66.0
Men, n (%)	21 (65.6)	…
Pregnancies, n (%)	9 (81.8)	…
BMI, kg/m^2	27.2±4.9	19.7–40.0
Age of onset of HTG, y	26.1±14.3	1–66
AP, n (%)	17 (51.3)	…
Age of onset of AP, y	28.9±13.3	10.0–58.0
Patients with recurrent AP, n (%)	8 (25.0)	…
Risk factors and comorbidities		
SBP, mm Hg	126.7±15.3	90.0–168.0
SDP, mm Hg	82.9±9.9	60.0–102.0
Smoking, n (%)	8 (25.0)	…
Obesity, n(%)	9 (28.1)	…
Diabetes mellitus, n (%)	10 (31.3)	…
Hypertension, n (%)	11 (34.4)	…
CHD, n (%)	4 (12.5)	…
Plasma lipids		
Total-C, mg/dL	305.9±122.3	153.0–565.0
HDL-C, mg/dL	25.7±12.7	9.0–60.0
TGs, mg/dL	2066.6±841.8	1019.0–4480.0
Non–HDL-C, mg/dL	266.1±109.5	141.0–519.0
Medications		
MCT oil, n (%)	5 (17.9)	…
Fish oil, n (%)	12 (42.9)	…
Fibrates, n (%)	13 (46.4)	…
Statins, n (%)	2 (7.1)	…
Glucose-lowering therapies, n (%)	3 (9.3)	
Metformin	1 (3.6)	…
Sulphonylurea	1 (3.6)	…
Insulin	1 (3.6)	…

Data are represented as mean±SD or n (%) as appropriate. AP indicates acute pancreatitis; BMI, body mass index; CHD, coronary heart disease; HDL-C, high-density lipoprotein cholesterol; HTG, hypertriglyceridemia; MCT, medium-chain triglyceride; SBP, systolic blood pressure; SDP, diastolic blood pressure; TG, triglyceride; and Total-C, total cholesterol.

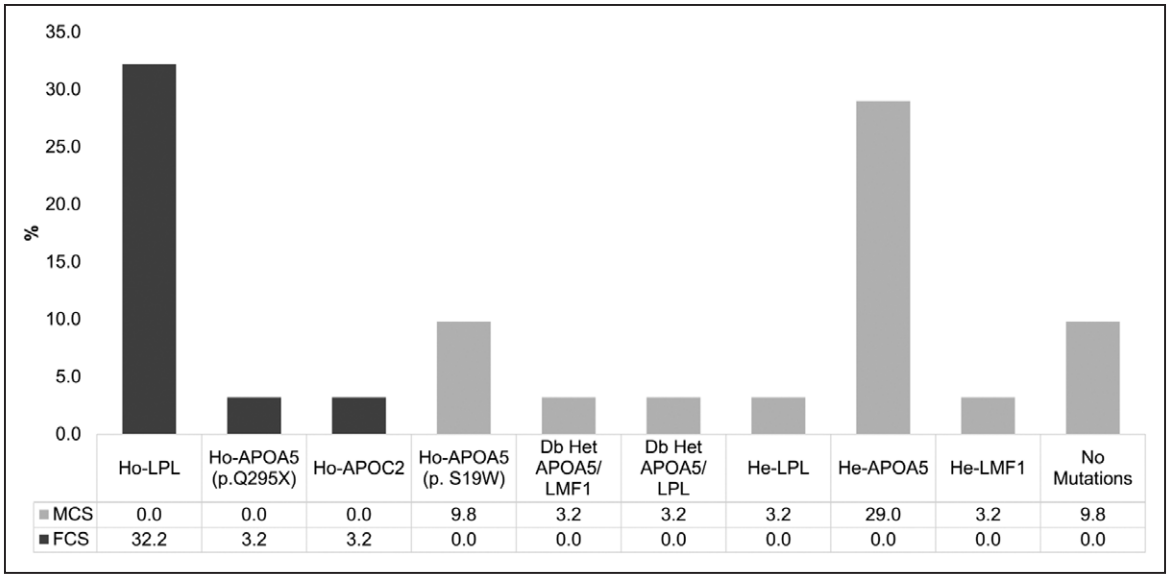

Figure 1. Comparison of genotype distribution in canonical genes in patients with familial chylomicronemia syndrome (FCS) and multifactorial chylomicronemia syndrome (MCS).
Percentage refers to the entire group of 32 patients with severe hypertriglyceridemia. *APOA5* indicates apolipoprotein A5; *APOC2*, apolipoprotein C2; Db Het, double heterozygous status; He, heterozygous status; Ho, homozygous status; *LMF1*, lipase maturation factor 1; and *LPL*, lipoprotein lipase.

(mean, 25.7±12.7 mg/dL). At baseline, in addition to a low caloric diet, 46.4% of patients were reported taking fibrates and 42.9% omega-3 fatty acids; 17.9% were using MCT oil for seasoning.

Genetic Profile of Study Patients

Based on the above-described criteria, 12 (37.5%) patients were classified as FCS, 19 (59.3%) as MCS, and 1 (3.1%) as genetically undetermined. Considering his genotype, this patient was not included for further analysis. The list of pathogenic and probably damaging variants detected in canonical genes, used for FCS and MCS groups classification, is shown in Table I in the online-only Data Supplement[26–31] and the comparison of genotype distribution between FCS and MCS in Figure 1.

Among FCS, 83.3% (n=10) of patients were carrier of biallelic mutations in *LPL*, whereas 8.3% (n=1) in *APOA5* gene. One patient was carrier of a nonsense mutation in *APOC2* in the homozygous state (8.3%; n=1). The family segregation of novel *LPL* variants and of those known but never associated to FCS is shown in the Figure I in the online-only Data Supplement. For the *LPL* p.I109T, p.Pro217Fs, and p.D277Fs variants, we identified additional 2, 1, and 1 heterozygous carriers, respectively, and all presented normal triglyceride levels. All together, these findings indicate the recessive nature of all these identified variants. Within family No. 2, the sister of the index case was found to be homozygous for the same *LPL* c.1019-2 A>T allele and showed severe hypertriglyceridemia.

Among MCS, 14 patients had causative mutations in *APOA5* gene: 9 were heterozygotes, 3 homozygotes for p.(S19W), and 2 patients double heterozygotes (one for *APOA5/LMF1* and one for *APOA5/LPL*). Moreover, 2 MCS patients were, respectively, heterozygotes for *LPL* and *LMF1* gene variants. Overall, 3 patients were negative for causative variations in any canonical genes.

The list of any other identified functional rare and low-frequency variants in canonical and noncanonical genes is reported in Table II in the online-only Data Supplement.[27–36] It is interesting to note that 3 of 14 *APOA5*-associated MCS patients had also rare harmful heterozygous variants in *CREB3L3* [p. (L233V) and p. (E239K)] and *GPD1* [p. (E59G) and p.(V74L)] genes. Moreover, 1 MCS patient showed a rare, nonsense mutation in *CREB3L3* gene (p.R241X; MAF, 4×10^{-5}). Overall, we found that MCS patients showed an increased accumulation of rare and low-frequency variants in canonical and noncanonical genes when compared with FCS individuals (30 versus 8). As a result, the median value of the unweighted rare-allele triglyceride score was significantly higher in MCS than in FCS patients (2 [1–6] versus 1 [0–2], respectively; P=0.005). For every increase of 1 unit of unweighted rare-allele triglyceride score, the percentage of patients with MCS rises as compared with FCS (P for linear association, 0.005; Figure 2A). We also found a higher accumulation of common triglyceride-rising alleles in FCS subjects (P for linear association, 0.02; Figure 2B). Despite this, stepwise regression analysis including both scores highlighted the independent ability of unweighted rare-allele triglyceride score in discriminating between FCS and MCS (OR, 4.9 [95% CI, 1.2–19.1]; P=0.02 of being MCS). Further, to confirm this observation, only the unweighted rare-allele triglyceride

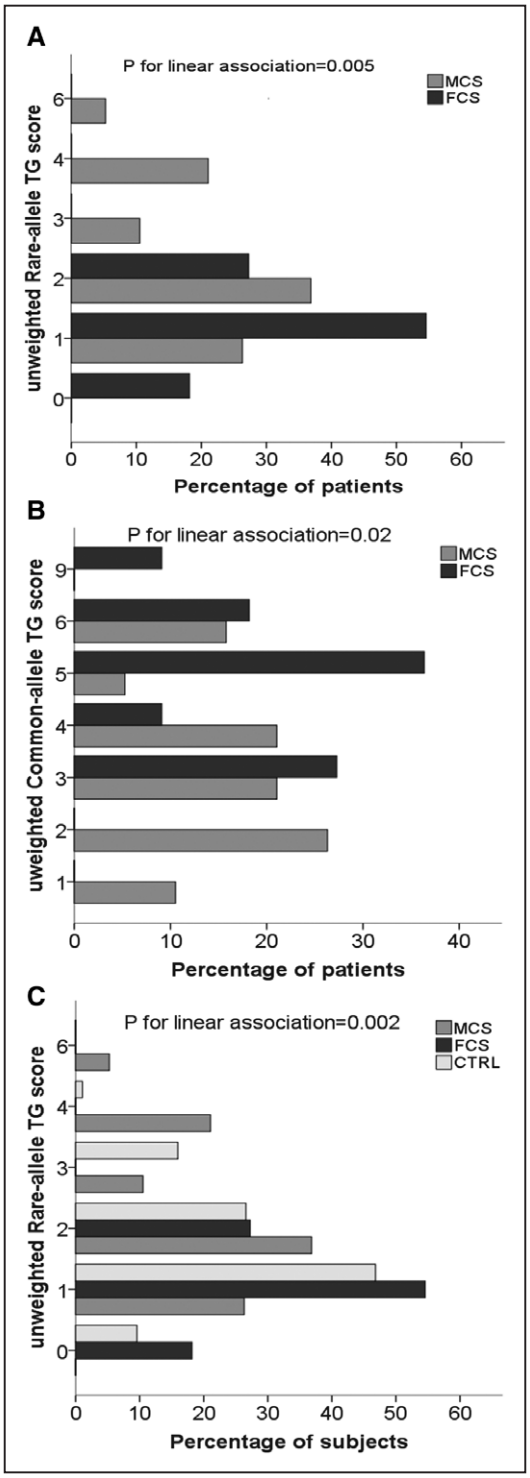

Figure 2. Prevalence of familial chylomicronemia syndrome (FCS), multifactorial chylomicronemia syndrome (MCS) diagnosis, and controls (CTRL) according to unweighted allele triglyceride (TG) scores.

A, We represented the unweighted rare-allele TG score according to FCS and MCS diagnosis. Data are represented as percentage of subjects for each 1-point increase in unweighted rare-allele TG score. Variants in canonical and noncanonical TGs raising genes were considered in both FCS and MCS patients. Biallelic mutations used for FCS diagnosis were excluded. **B**, We represented the unweighted common-allele TG score according to (*Continued*)

score was tested in the normotriglyceridemic controls. In this analysis, MCS patients exhibited higher rare-allele triglyceride score values when compared with controls (2 [1–6] versus 1 [0–4]; *P*=0.017). No differences were found in the comparison between FCS and controls. For every 1-unit increase in the number of rare and low-frequency risk alleles, the percentage of patients with MCS significantly raised as compared with FCS and controls (*P* for linear association, 0.002; Figure 2C). Therefore, MCS patients showed 2-fold higher risk to accumulate rare triglyceride risk alleles when compared with controls (OR, 2.2 [95% CI, 1.3–3.6]; *P*=0.001; data not shown).

Comparison of Clinical Outcomes in FCS and MCS

No differences in sex were found between the 2 groups. The observational period was significantly longer in FCS than in MCS (59 [2–204] versus 27 [4–95] months; *P*=0.04, respectively). Similarly, the age of onset of hypertriglyceridemia was significantly earlier in FCS than in MCS (17.6±6.9 versus 30.6±15.5 years; *P*=0.011, respectively). Atherosclerotic cardiovascular disease events were reported, respectively, in 3 MCS (15.8%) and in 1 (9.1%) FCS patient, and this difference was not statistically significant.

Table 2 shows changes in lipid levels during the observational period in FCS and MCS patients. At baseline, triglycerides levels were comparable between groups, but a larger proportion of FCS patients were taking triglyceride-lowering medications. In fact, 90.9% of FCS patients were taking fish oil, 81.8% fibrates, and the 45.5% were using MCT oil while these figures were 6.3%, 18.8%, and 0%, respectively, in the MCS group (*P*<0.001, *P*=0.001, *P*=0.003 for differences). Among MCS, 2 patients were also taking statins.

During the observational period, the average triglyceride level remained significantly higher (*P*=0.01) and HDL-C lower (*P*<0.001) in FCS as compared with MCS. Moreover, the mean achieved nadir triglyceride level was 366.3±358.5 mg/dL in MCS and 759.6±385.8 mg/dL in FCS (*P*=0.007 for comparison between groups). This occurred despite FCS patients having received a more intense treatment with most of them taking a combination of fibrates (90.9%), MCT oil (64.5%), omega-3 fatty acids (81.8%), while a smaller percentage of MCS patients were treated with lipid-lowering medications (Table 2).

The individual on-treatment levels of triglycerides during the observational period in FCS and MCS patients

Figure 2 Continued. FCS and MCS diagnosis. Data are represented as percentage of subjects for each 1-point increase in unweighted common-allele TG score. **C**, We represented the unweighted rare-allele TG score according to FCS, MCS diagnosis, and CTRL. Data are represented as percentage of subjects for each 1-point increase in unweighted common-allele TG score. A *P* value <0.05 is considered as significant.

Table 2. Plasma Lipid Changes and Treatments During Follow-Up in Hypertriglyceridemia Patients Classified as FCS and MCS

	FCS (n=12)			MCS (n=19)					
	Baseline	On Treatment*	Best Result†	Baseline	On Treatment*	Best Result†	$P_{Baseline}$	$P_{Follow-Up}$	$P_{Best\ Result}$
Plasma lipids									
Total-C, mg/dL	239.9±67.8	246.0±90.9	143.5±30.6	350.2±136.2	265.4±79.5	199.1±73.8	0.02	NS	0.02
HDL-C, mg/dL	20.0±15.7	15.8±4.6	16.0±4.1	30.9±7.7	32.8±9.3	37.8±8.2	0.06	<0.001	<0.001
TGs, mg/dL	2202.7±588.0	1964.8±951.5	759.6±385.8	1967.4±991.4	1047.3±577.7	366.3±358.5	NS	0.002	0.007
Non–HDL-C, mg/dL	226.2±62.6	215.1±65.5	192.2±30.5	298.8±137.6	235.0±81.5	160.3±71.4	NS	NS	NS
TG <500, n (%)	…	…	4 (33.3)	…	…	16 (84.2)	…	…	0.004
TG <700, n (%)	…	0		…	7 (36.8)	…	…	0.014	…
Medications									
MCT oil, n (%)	5 (45.5)	…	6 (64.5)	0	…	2 (12.5)	0.003	…	0.011
Fish oil, n (%)	10 (90.9)	…	9 (81.8)	1 (6.3)	…	7 (38.9)	<0.001	…	0.024
Fibrates, n (%)	9 (81.8)	…	10 (90.9)	3 (18.8)	…	12 (66.7)	0.001	…	NS
Statins, n (%)	0	…	1 (9.1)	2 (12.5)	…	9 (50.0)	NS	…	0.025
Glucose-lowering therapies, n (%)									
Metformin	1 (9.1)	…	2 (18.2)	0	…	4 (22.2)	NS	…	NS
Sulphonylurea	1 (9.1)	…	1 (9.1)	0	…	0	NS	…	NS
Insulin	0	…	0	2 (12.5)	…	3 (18.8)	NS	…	NS

Data are represented as mean±SD or n (%) as appropriate.

For the definition of FCS and MCS, refer to the text. FCS indicates familial chylomicronemia syndrome; HDL-C, high-density lipoprotein cholesterol; MCS, multifactorial chylomicronemia syndrome; MCT, medium-chain triglyceride; NS, nonsignificant; TG, triglyceride; and Total-C, total cholesterol.

*Data refer to average values reported during the observational period.

†Data refer to the best TG values reported during the observational period.

are presented in Figure II in the online-only Data Supplement. It is notable that 36.8% of MCS patients achieved an on-treatment average triglyceride level <700 mg/dL, whereas none of FCS patients reached this cutoff (*P*=0.014; Table 2).

As shown in Figure 3, the overall prevalence of AP was 75% in FCS and 37% in MCS (OR, 5.1 [95% CI, 1.03–25.6]; *P*=0.046). The cumulative number of episodes for FCS and MCS were, respectively, 23 versus 11, but no differences were found in the recurrences of AP between groups. Therefore, we estimated an overall incidence rate of AP of 42 per 1000 person-years in FCS and 13 per 1000 person-years in MCS (*P*<0.001). No differences were found in the age of onset of AP between groups (25.1±8.2 years for FCS and 31.8±12.8 years for MCS; *P*=nonsignificant).

Evaluating predictors of FCS, we found that age of onset of triglycerides later in life, higher on-treatment HDL-C or nadir HDL-C values, and the achievement of nadir triglycerides <500 mg/dL were suggestive of MCS genotype (Table 3A). Conversely, history of AP, the number of episodes of AP experienced during life, higher on-treatment triglycerides, and nadir triglyceride levels >500 mg/dL were significant predictors of the FCS genotype

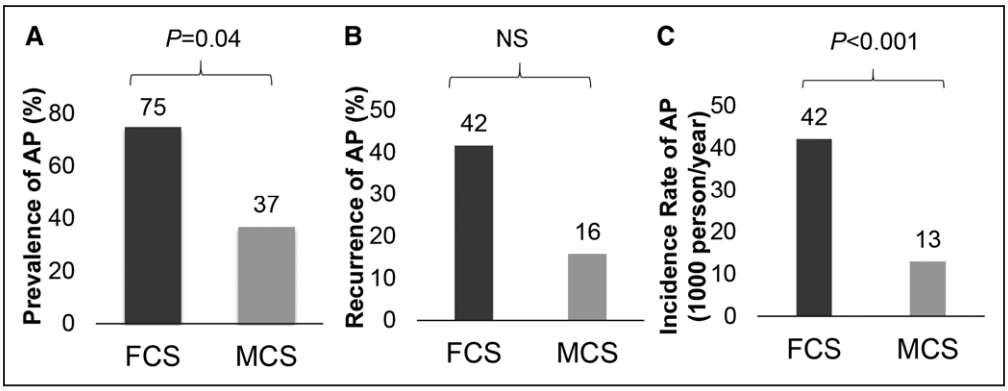

Figure 3. Acute pancreatitis (AP) outcomes in patients with familial chylomicronemia syndrome (FCS) and multifactorial chylomicronemia syndrome (MCS).

A, Prevalence of AP at baseline; data are shown as percentage of subjects according to the size of each group. **B**, Occurrence of AP (>1 event); data are shown as percentage of subjects according to the size of each group. **C**, Estimated overall incidence rate of AP per 1000 person/y observed in each group. A *P* value <0.005 was considered as significant. NS indicates nonsignificant.

Table 3. Predictors of Chylomicronemia Syndromes

Covariate	OR	CI	*P* Value
MCS			
Age of onset of TGs, y	1.113	1.012–1.225	0.028
On-treatment HDL-C, mg/dL*	1.377	1.085–1.748	0.009
HDL-C best results, mg/dL†	1.541	1.020–2.330	0.040
TG best results <500 mg/dL, %†	10.66	1.909–59.615	0.007
FCS			
AP, %	5.143	1.033–25.602	0.046
No. of episodes of AP, n	2.029	1.014–4.059	0.045
On-treatment TGs, mg/dL*	1.002	1.000–1.003	0.011
TG best results <500 mg/dL, %†	0.107	0.019–0.611	0.012

As the recurrence of AP, age of onset of AP, and on-treatment TGs <700 mg/dL were not significantly related with genetic chylomicronemia syndromes in our cohort, we did not include these variables in the present analysis. A *P* value <0.05 was considered as significant. AP indicates acute pancreatitis; FCS, familial chylomicronemia syndrome; HDL-C, high-density lipoprotein cholesterol; MCS, multifactorial chylomicronemia syndrome; OR, odds ratio; and TG, triglyceride.

*Data refer to average values reported during the observational period.

†Data refer to the best TG values reported during the observational period.

(Table 3B). After adjusting for all significant covariates by stepwise logistic regression, nadir HDL-C was the best predictor of MCS (OR, 1.5 [95% CI, 1.020–2.330]; *P*=0.04; data not shown).

Comparison of Clinical Characteristics of LPL Versus Non-LPL FCS

In the 12 subjects with confirmed FCS, we further evaluated whether there was any clinical difference according to *LPL* or non-*LPL* genotype. At baseline, triglycerides were not different between the groups, as well as the age of onset of hypertriglyceridemia or AP (data not shown). During follow-up, the 2 non-*LPL* FCS patients experienced a much better, although not significant, control of hypertriglyceridemia compared with *LPL* FCS patients as demonstrated by the lower average triglyceride levels (950.8±126.1 versus 2167.5±911.3 mg/dL, respectively; *P*=nonsignificant) and lower nadir of triglyceride levels (317.0±25.3 versus 848.1±360.0 mg/dL, respectively; *P*=nonsignificant). In addition, whereas only 20% of *LPL* FCS patients achieved a nadir triglycerides level of <500 mg/dL, the whole subgroup (100%) of non-*LPL* FCS patients reached this target (*P*=0.028 for comparison between the groups). These results were achieved despite no differences in triglyceride-lowering medication use (excluding statins). In both FCS groups, none achieved an on-treatment triglyceride level <700 mg/dL. Finally, no differences were found in the prevalence of AP or the recurrence of episodes, as well as the incident rates of AP (data not shown).

DISCUSSION

In the present study, we have compared the genetic profiles and the natural history of 2 genetically determined chylomicronemia syndromes (FCS and MCS).[2,6,9] To this aim, we molecularly characterized a cohort of patients with persistent triglyceride >1000 mg/dL despite therapy, with or without history of AP excluding any major nongenetic hypertriglyceridemia contributing factors.

Across the study population, 37.5% were classified as FCS as carriers of biallelic, rare mutations and 59.3% as MCS due to heterozygosity or homozygosity for pathogenic and nonpathogenic variants in canonical and noncanonical genes. Because 1 patient was genetically undetermined for all sequenced genes, his data were not included in the present study.

Despite the presence of few rare and low-frequency SNPs in FCS, the burden of polygenic rare variants was significantly higher in MCS than in FCS. Indeed, we highlighted the independent ability of unweighted rare-allele triglyceride score to predict MCS diagnosis. These figures are in sharp contrast with previous reports indicating that severe hypertriglyceridemia in adults is rarely associated with rare variants. In particular, Dron et al[2] in a large cohort of 563 patients with severe hypertriglyceridemia (defined as triglyceride ≥885 mg/dL) found that only 1.1% patients had biallelic rare variants while the majority (52.6%) was genetically unclassified. Moreover, in a Spanish cohort with hypertriglyceridemia,[31] 4.2% of patients were homozygous or compound heterozygous for pathogenic mutations and 14.4% were heterozygous carriers of rare variants while these variants where absent in controls. The most reasonable explanation of these discrepancies relies on the differences in the populations enrolled in these 2 studies; in fact, we have chosen stricter entry criteria, possibly enriching our sample of genetic causes, both polygenic and monogenic. On average, our patients were younger, showed lower body mass index, lower prevalence of diabetes mellitus, as well as persistent elevation of plasma triglycerides. Indeed, it has been recently shown that fasting triglyceride levels >10 mmol/L (885 mg/dL) on multiple occasions, refractory to standard triglyceride-lowering therapies, a young age at onset, the lack of secondary factors, and a history of episodes of AP are powerful predictors of FCS.[3]

In agreement with previous reports,[6] the most common molecular defect in FCS patients was represented by rare loss-of-function mutations in *LPL* gene. We detected 9 *LPL* mutations, 3 of which are already known to be causative of FCS[26] (https://www.omim.org/entry/609708), whereas 4 were novel and 2 known but never associated to FCS phenotype. We did not perform in vitro functional studies of novel *LPL* variants (p.I109T, p.P217Fs, p.D277Fs, and c.1019-2A>T), but their family segregation demonstrated that when present at heterozygous state, they were not associated with significant elevation of plasma triglyceride, thus confirming their recessive nature. In FCS patients, we have also identified 1 pathogenic mutation in *APOA5* gene [p.(Q295X)] and a rare nonsense mutation in *APOC2* gene (p.Q92X).[8] In

any case, the coexistence of double dose of molecular defects impairing the clearance of TRLs may well explain the extreme elevation of triglyceride in FCS.

Conversely, the genetic profile of patients with MCS was profoundly different. In fact, 73.7% (n=14) of them showed heterozygous causative variants in *APOA5* gene associated with various combinations of pathogenic and nonpathogenic variants in other triglyceride-raising genes. Interestingly, in *APOA5*-based MCS subjects, we observed an enrichment of causative variants in *CREB3L3* and *GPD1* genes and a significantly higher polygenic burden for rare and low-frequency variants in the 18 triglyceride-raising genes sequenced. Overall, these findings are consistent with the emerging model indicating that severe hypertriglyceridemia phenotype may be caused by the contribution of multiple alleles with large or minor effects interacting each others.[3] However, a clear explanation of mechanisms linking the increased burden of small-effect variants in triglyceride genes with the extreme elevation of plasma triglycerides is not available. Previous observations have highlighted the importance of alterations in the kinetic of LPL activation. In fact, Marmontel et al[37] have reported that the extended assessment of post heparin LPL activity after 60 minutes after heparin injection revealed abnormality in both LPL lipolysis ability and availability in MCS patients. Speculatively, this finding may be in line with our observation, which demonstrated that *APOA5* variants were the predominant single genetic defect in MCS. In fact, it has been well demonstrated that APOA5 is crucial in regulating LPL, and its dysfunction may impact on LPL activity and availability.[38] However, additional investigations are warranted to better understand the pathogenesis of severe and persistent elevation of triglycerides in MCS.

The second main result of our study was the observation of a remarkable difference in the natural history of patients classified as FCS or MCS. Even though there was no significant difference between the 2 groups in baseline triglyceride levels, MCS showed a better response to triglyceride-lowering therapies during a median observational period of >27 months despite a lower intensification of treatment. Indeed, the latter achieved a mean nadir level of triglycerides much lower than FCS. In addition, 84.2% of MCS patients achieved at least once a triglyceride level <500 mg/dL, whereas this cutoff value was reached by only 33.3% of FCS patients. In agreement with previous studies,[39] we found that *LPL*-driven FCS was the genotype most resistant to therapies. In fact, while all non-*LPL* FCS patients experienced, at least once, a triglyceride value <500 mg/dL during follow-up, this result was achieved in only 20% of *LPL* FCS.

The differences in the time course change of triglyceride levels were paralleled with an increased risk of complications, namely AP, which was found to be higher in FCS than in MCS. Also Paquette et al[40] have recently reported in a cross-sectional analysis that FCS patients have higher frequency of AP than MCS. However, because of the longitudinal nature of our study, we have been able to expand these findings estimating that FCS showed a 3-fold increased risk of incident AP events in comparison with MCS patients. The higher incident rate observed in FCS as compared with MCS, as well as the higher total number of episode of AP, could be explained by the increased magnitude of long-term exposure to hypertriglyceridemia in FCS patients. Overall, our findings further emphasize the clinical importance of differentiating these 2 types of genetically determined severe hypertriglyceridemia.

Our results might have some clinical implications. They are in favor of the concept that in presence of a patient with persistent, severe hypertriglyceridemia (triglyceride >1000 mg/dL), it may be reasonable to carry efforts in characterizing monogenic forms by molecular testing. By this approach, one might identify patients who are at higher risk of triglyceride-related complications such as AP, thus requiring a timely and more intensive lipid-lowering intervention. This may be in analogy with the extremely high levels of LDL (low-density lipoprotein) cholesterol presenting as suspected familial hypercholesterolemia. It has been shown that the familial hypercholesterolemia phenotype may have both monogenic and polygenic bases,[41] but there are evidences that polygenic familial hypercholesterolemia is associated with better responsiveness to LDL-lowering medications and lower risk of atherosclerotic complications.[41]

Our study has some limitations to be acknowledged. The study cohort was small in size, but this may well represent the rarity of severe hypertriglyceridemia phenotype. In fact, only ≈0.1% of patients attending our Lipid Clinic were considered for enrollment into the study, and this frequency is close to that reported for severe hypertriglyceridemia in the general population. Another important limitation is that, in evaluating the polygenic burden, we did not consider all potentially triglyceride-raising variants. In a recent study using next-generation sequencing, 73 genes and 185 SNPs were genotyped.[2] However, it must be pointed out that genes considered in the present study showed the largest influence on triglyceride levels as reported by several Genome-Wide Association Studies.[14,15,33] Nevertheless, this leaves the possibility that our estimations of triglyceride-raising genetic risk score may be incomplete. However, it is remarkable that in our cohort, only a minority of patients with severe hypertriglyceridemia remained genetically uncharacterized (3.1%). In addition, we did not perform measurements of LPL mass and activity, as well as of functionality of novel LPL variants, thus hampering the pathogenetic interpretations of genotyping. Despite during follow-up the intensity of triglyceride-lowering therapies was maximized, we were unable to evaluate the compliance to treatment. Finally, the adjudication of AP was not based on a well-standardized protocol but was

only based on patients' medical records. However, one of us (L.D.) carried out any possible effort in reviewing all discharged record, as well as in contacting taking care physicians.

In summary, our data indicate that FCS, due to biallelic mutations in *LPL*, was the most common genotype underlying severe hypertriglyceridemia. Heterozygosity for *APOA5* variants in combination with high polygenic burden was the most frequent genotype of MCS. FCS expressed the most severe clinical phenotype as determined by resistance to triglyceride-lowering medications and higher occurrence of AP. Overall, these results highlighted the importance of diagnosing patients with severe hypertriglyceridemia and the need of novel therapies to treat the most severe genetic forms.

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Disclosures

None.

REFERENCES

1. Johansen CT, Kathiresan S, Hegele RA. Genetic determinants of plasma triglycerides. *J Lipid Res.* 2011;52:189–206. doi: 10.1194/jlr.R009720
2. Dron JS, Wang J, Cao H, McIntyre AD, Iacocca MA, Menard JR, Movsesyan I, Malloy MJ, Pullinger CR, Kane JP, et al. Severe hypertriglyceridemia is primarily polygenic. *J Clin Lipidol.* 2019;13:80–88. doi: 10.1016/j.jacl.2018.10.006
3. Moulin P, Dufour R, Averna M, Arca M, Cefalù AB, Noto D, D'Erasmo L, Di Costanzo A, Marçais C, Alvarez-Sala Walther LA, et al. Identification and diagnosis of patients with familial chylomicronaemia syndrome (FCS): expert panel recommendations and proposal of an "FCS score". *Atherosclerosis.* 2018;275:265–272. doi: 10.1016/j.atherosclerosis.2018.06.814
4. Christian JB, Arondekar B, Buysman EK, Johnson SL, Seeger JD, Jacobson TA. Clinical and economic benefits observed when follow-up triglyceride levels are less than 500 mg/dL in patients with severe hypertriglyceridemia. *J Clin Lipidol.* 2012;6:450–461. doi: 10.1016/j.jacl.2012.08.007
5. Zafrir B, Jubran A, Hijazi R, Shapira C. Clinical features and outcomes of severe, very severe, and extreme hypertriglyceridemia in a regional health service. *J Clin Lipidol.* 2018;12:928–936. doi: 10.1016/j.jacl.2018.03.086
6. Brahm AJ, Hegele RA. Chylomicronaemia–current diagnosis and future therapies. *Nat Rev Endocrinol.* 2015;11:352–362. doi: 10.1038/nrendo.2015.26
7. Brahm A, Hegele RA. Hypertriglyceridemia. *Nutrients.* 2013;5:981–1001. doi: 10.3390/nu5030981
8. Wolska A, Dunbar RL, Freeman LA, Ueda M, Amar MJ, Sviridov DO, Remaley AT. Apolipoprotein C-II: New findings related to genetics, biochemistry, and role in triglyceride metabolism. *Atherosclerosis.* 2017;267:49–60. doi: 10.1016/j.atherosclerosis.2017.10.025
9. Hegele RA, Ginsberg HN, Chapman MJ, Nordestgaard BG, Kuivenhoven JA, Averna M, Borén J, Bruckert E, Catapano AL, Descamps OS, et al; European Atherosclerosis Society Consensus Panel. The polygenic nature of hypertriglyceridaemia: implications for definition, diagnosis, and management. *Lancet Diabetes Endocrinol.* 2014;2:655–666. doi: 10.1016/S2213-8587(13)70191-8
10. Rabacchi C, Pisciotta L, Cefalù AB, Noto D, Fresa R, Tarugi P, Averna M, Bertolini S, Calandra S. Spectrum of mutations of the LPL gene identified in Italy in patients with severe hypertriglyceridemia. *Atherosclerosis.* 2015;241:79–86. doi: 10.1016/j.atherosclerosis.2015.04.815
11. Lewis GF, Xiao C, Hegele RA. Hypertriglyceridemia in the genomic era: a new paradigm. *Endocr Rev.* 2015;36:131–147. doi: 10.1210/er.2014-1062
12. Yuan G, Al-Shali KZ, Hegele RA. Hypertriglyceridemia: its etiology, effects and treatment. *CMAJ.* 2007;176:1113–1120. doi: 10.1503/cmaj.060963
13. Di Costanzo A, D'Erasmo L, Polimeni L, Baratta F, Coletta P, Di Martino M, Loffredo L, Perri L, Ceci F, Montali A, et al. Non-alcoholic fatty liver disease and subclinical atherosclerosis: a comparison of metabolically- versus genetically-driven excess fat hepatic storage. *Atherosclerosis.* 2017;257:232–239. doi: 10.1016/j.atherosclerosis.2016.12.018
14. Johansen CT, Hegele RA. Allelic and phenotypic spectrum of plasma triglycerides. *Biochim Biophys Acta.* 2012;1821:833–842. doi: 10.1016/j.bbalip.2011.10.007
15. Johansen CT, Wang J, Lanktree MB, McIntyre AD, Ban MR, Martins RA, Kennedy BA, Hassell RG, Visser ME, Schwartz SM, et al. An increased burden of common and rare lipid-associated risk alleles contributes to the phenotypic spectrum of hypertriglyceridemia. *Arterioscler Thromb Vasc Biol.* 2011;31:1916–1926. doi: 10.1161/ATVBAHA.111.226365
16. Cefalù AB, Spina R, Noto D, Ingrassia V, Valenti V, Giammanco A, Fayer F, Misiano G, Cocorullo G, Scrimali C, et al. Identification of a novel LMF1 nonsense mutation responsible for severe hypertriglyceridemia by targeted next-generation sequencing. *J Clin Lipidol.* 2017;11:272–281.e8. doi: 10.1016/j.jacl.2017.01.003
17. Pulignani S, Vecoli C, Borghini A, Foffa I, Ait-Alì L, Andreassi MG. Targeted next-generation sequencing in patients with non-syndromic congenital heart disease. *Pediatr Cardiol.* 2018;39:682–689. doi: 10.1007/s00246-018-1806-y
18. Di Costanzo A, Belardinilli F, Bailetti D, Sponziello M, D'Erasmo L, Polimeni L, Baratta F, Pastori D, Ceci F, Montali A, et al. Evaluation of polygenic determinants of Non-Alcoholic Fatty Liver Disease (NAFLD) by a candidate genes resequencing strategy. *Sci Rep.* 2018;8:3702. doi: 10.1038/s41598-018-21939-0
19. Yang H, Wang K. Genomic variant annotation and prioritization with ANNOVAR and wANNOVAR. *Nat Protoc.* 2015;10:1556–1566. doi: 10.1038/nprot.2015.105
20. Panoutsopoulou K, Tachmazidou I, Zeggini E. In search of low-frequency and rare variants affecting complex traits. *Hum Mol Genet.* 2013;22(R1):R16–R21. doi: 10.1093/hmg/ddt376
21. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, et al; ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med.* 2015;17:405–424. doi: 10.1038/gim.2015.30
22. Jørgensen AB, Frikke-Schmidt R, West AS, Grande P, Nordestgaard BG, Tybjærg-Hansen A. Genetically elevated non-fasting triglycerides and calculated remnant cholesterol as causal risk factors for myocardial infarction. *Eur Heart J.* 2013;34:1826–1833. doi: 10.1093/eurheartj/ehs431
23. Pennacchio LA, Olivier M, Hubacek JA, Cohen JC, Cox DR, Fruchart JC, Krauss RM, Rubin EM. An apolipoprotein influencing triglycerides in humans and mice revealed by comparative sequencing. *Science.* 2001;294:169–173. doi: 10.1126/science.1064852
24. Pennacchio LA, Olivier M, Hubacek JA, Krauss RM, Rubin EM, Cohen JC. Two independent apolipoprotein A5 haplotypes influence human plasma triglyceride levels. *Hum Mol Genet.* 2002;11:3031–3038. doi: 10.1093/hmg/11.24.3031
25. Wang J, Lin M, Crenshaw A, Hutchinson A, Hicks B, Yeager M, Berndt S, Huang WY, Hayes RB, Chanock SJ, et al. High-throughput single nucleotide polymorphism genotyping using nanofluidic dynamic arrays. *BMC Genomics.* 2009;10:561.
26. Gupta N, Moore D, Hooper AJ, Burnett JR, Metz M. Pancreatitis in a child with lipemia due to novel lipoprotein lipase mutations. *J Pediatr Gastroenterol Nutr.* 2010;50:457–459. doi: 10.1097/MPG.0b013e3181b64407
27. Evans D, Aberle J, Beil FU. Resequencing the apolipoprotein A5 (APOA5) gene in patients with various forms of hypertriglyceridemia. *Atherosclerosis.* 2011;219:715–720. doi: 10.1016/j.atherosclerosis.2011.09.030
28. Di Filippo M, Marçais C, Charrière S, Marmontel O, Broyer M, Delay M, Merlin M, Nollace A, Valéro R, Lagarde M, et al. Post-heparin LPL

activity measurement using VLDL as a substrate: a new robust method for routine assessment of plasma triglyceride lipolysis defects. *PLoS One.* 2014;9:e99721. doi: 10.1371/journal.pone.0099721
29. Johansen CT, Wang J, McIntyre AD, Martins RA, Ban MR, Lanktree MB, Huff MW, Péterfy M, Mehrabian M, Lusis AJ, et al. Excess of rare variants in non-genome-wide association study candidate genes in patients with hypertriglyceridemia. *Circ Cardiovasc Genet.* 2012;5:66–72. doi: 10.1161/CIRCGENETICS.111.960864
30. Buonuomo PS, Rabacchi C, Macchiaiolo M, Trenti C, Fasano T, Tarugi P, Bartuli A, Bertolini S, Calandra S. Incidental finding of severe hypertriglyceridemia in children. Role of multiple rare variants in genes affecting plasma triglyceride. *J Clin Lipidol.* 2017;11:1329–1337.e3. doi: 10.1016/j.jacl.2017.08.017
31. De Castro-Orós I, Civeira F, Pueyo MJ, Mateo-Gallego R, Bolado-Carrancio A, Lamíquiz-Moneo I, Álvarez-Sala L, Fabiani F, Cofán M, Cenarro A, et al. Rare genetic variants with large effect on triglycerides in subjects with a clinical diagnosis of familial vs nonfamilial hypertriglyceridemia. *J Clin Lipidol.* 2016;10:790–797. doi: 10.1016/j.jacl.2016.02.010
32. Guo T, Yin RX, Wu J, Lin QZ, Shi GY, Shen SW, Sun JQ, Li H, Lin WX, Yan DZ. Association of the angiopoietin-like protein 8 rs2278426 polymorphism and several environmental factors with serum lipid levels. *Mol Med Rep.* 2015;12:3285–3296. doi: 10.3892/mmr.2015.3825
33. Johansen CT, Wang J, Lanktree MB, Cao H, McIntyre AD, Ban MR, Martins RA, Kennedy BA, Hassell RG, Visser ME, et al. Excess of rare variants in genes identified by genome-wide association study of hypertriglyceridemia. *Nat Genet.* 2010;42:684–687. doi: 10.1038/ng.628
34. Surendran RP, Visser ME, Heemelaar S, Wang J, Peter J, Defesche JC, Kuivenhoven JA, Hosseini M, Péterfy M, Kastelein JJ, et al. Mutations in LPL, APOC2, APOA5, GPIHBP1 and LMF1 in patients with severe hypertriglyceridaemia. *J Intern Med.* 2012;272:185–196. doi: 10.1111/j.1365-2796.2012.02516.x
35. Carlquist JF, McKinney JT, Horne BD, Camp NJ, Cannon-Albright L, Muhlestein JB, Hopkins P, Clarke JL, Mower CP, Park JJ, et al. Common Variants in 6 lipid-related genes discovered by high-resolution DNA melting analysis and their association with plasma lipids. *J Clin Exp Cardiolog.* 2011;2:pii: 2155-9880-2-138.
36. Lee JH, Giannikopoulos P, Duncan SA, Wang J, Johansen CT, Brown JD, Plutzky J, Hegele RA, Glimcher LH, Lee AH. The transcription factor cyclic AMP-responsive element-binding protein H regulates triglyceride metabolism. *Nat Med.* 2011;17:812–815. doi: 10.1038/nm.2347
37. Marmontel O, Di Filippo M, Marcais C, Nony S, Dumoux M, Serveaux-Dancer M, Caussy C, Charrière S, Moulin P. Alterations in plasma triglycerides lipolysis in patients with history of multifactorial chylomicronemia. *Atherosclerosis.* 2017;265:22–28. doi: 10.1016/j.atherosclerosis.2017.07.030
38. Kersten S. Physiological regulation of lipoprotein lipase. *Biochim Biophys Acta.* 2014;1841:919–933. doi: 10.1016/j.bbalip.2014.03.013
39. Hegele RA, Berberich AJ, Ban MR, Wang J, Digenio A, Alexander VJ, D'Erasmo L, Arca M, Jones A, Bruckert E, et al. Clinical and biochemical features of different molecular etiologies of familial chylomicronemia. *J Clin Lipidol.* 2018;12:920–927.e4. doi: 10.1016/j.jacl.2018.03.093
40. Paquette M, Bernard S, Hegele RA, Baass A. Chylomicronemia: differences between familial chylomicronemia syndrome and multifactorial chylomicronemia. *Atherosclerosis.* 2019;283:137–142. doi: 10.1016/j.atherosclerosis.2018.12.019
41. Futema M, Bourbon M, Williams M, Humphries SE. Clinical utility of the polygenic LDL-C SNP score in familial hypercholesterolemia. *Atherosclerosis.* 2018;277:457–463. doi: 10.1016/j.atherosclerosis.2018.06.006