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Searching for new genes associated with the familial hypercholesterolemia phenotype using whole-genome sequencing and machine learning

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Abstract. One of the most common congenital metabolic disorders is familial hypercholesterolemia. Familial hypercholesterolemia is a condition caused by a type of genetic defect leading to a decreased rate of removal of low-density lipoproteins from the bloodstream and a pronounced increase in the blood level of total cholesterol. This disease leads to the early development of cardiovascular diseases of atherosclerotic etiology. Familial hypercholesterolemia is a monogenic disease that is predominantly autosomal dominant. Rare pathogenic variants in the *LDLR* gene are present in 75–85 % of cases with an identified molecular genetic cause of the disease, and variants in other genes (*APOB*, *PCSK9*, *LDLRAP1*, *ABCG5*, *ABCG8*, and others) occur at a frequency of < 5 % in this group of patients. A negative result of genetic screening for pathogenic variants in genes of the low-density lipoprotein receptor and its ligands does not rule out a diagnosis of familial hypercholesterolemia. In 20–40 % of cases, molecular genetic testing fails to detect changes in the above genes. The aim of this work was to search for new genes associated with the familial hypercholesterolemia phenotype by modern high-tech methods of sequencing and machine learning. On the basis of a group of patients with familial hypercholesterolemia (enrolled according to the Dutch Lipid Clinic Network Criteria and including cases confirmed by molecular genetic analysis), decision trees were constructed, which made it possible to identify cases in the study population that require additional molecular genetic analysis. Five probands were identified as having the severest familial hypercholesterolemia without pathogenic variants in the studied genes and were analyzed by whole-genome sequencing on the HiSeq 1500 platform (Illumina). The whole-genome sequencing revealed rare variants in three out of five analyzed patients: a heterozygous variant (rs760657350) located in a splicing acceptor site in the *PLD1* gene (c.2430-1G>A), a previously undescribed single-nucleotide deletion in the *SIDT1* gene [c.2426del (p.Leu809CysfsTer2)], new missense variant c.10313C>G (p.Pro3438Arg) in the *LRP1B* gene, and single-nucleotide deletion variant rs753876598 [c.165del (p.Ser56AlafsTer11)] in the *CETP* gene. All these variants were found for the first time in patients with a clinical diagnosis of familial hypercholesterolemia. Variants were identified that may influence the formation of the familial hypercholesterolemia phenotype.
Key words: familial hypercholesterolemia; whole-genome sequencing; machine learning; *SIDT1*; *LRP1B*; *PLD1*; *CETP*.

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Поиск новых генов, ассоциированных с фенотипом семейной гиперхолестеринемии, методами полногеномного секвенирования и машинного обучения

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Аннотация. Одним из наиболее распространенных врожденных метаболических нарушений является семейная гиперхолестеринемия. Это заболевание приводит к раннему развитию сердечно-сосудистых заболеваний атеросклеротического генеза. Семейная гиперхолестеринемия относится к моногенным заболеваниям с пре-

имущественно аутосомно-доминантным типом наследования. Редкие патогенные варианты в гене *LDLR* определяются в 75–85 % случаев у пациентов с выявленной молекулярно-генетической причиной заболевания, варианты в других генах встречаются с частотой менее 5 % (*APOB*, *PCSK9*, *LDLRAP1*, *ABCG5*, *ABCG8* и др.). Отрицательный результат генетического скрининга патогенных вариантов генов рецептора липопротеинов низкой плотности и его лигандов не исключает диагноз «семейная гиперхолестеринемия». В 20–40 % случаев при молекулярно-генетическом исследовании не удается определить изменения в вышеназванных генах. Цель настоящей работы – поиск новых генов, ассоциированных с фенотипом семейной гиперхолестеринемии, с использованием современных высокотехнологичных методов секвенирования и машинного обучения. На основании выборки пациентов с семейной гиперхолестеринемией, сформированной по критериям Dutch Lipid Clinic Network и включающей случаи заболевания, подтвержденные молекулярно-генетическим анализом, построены решающие деревья, которые позволили выделить из выборки случаи, требующие дополнительного молекулярно-генетического анализа. Определены пять пробандов с наиболее тяжелым течением семейной гиперхолестеринемии без патогенных вариантов в изученных генах для проведения полногеномного секвенирования на платформе HiSeq 1500 (Illumina). При выполнении полногеномного секвенирования у трех из пяти обследованных пациентов найдены редкие варианты: гетерозиготный вариант (rs760657350), локализованный в акцепторном сайте сплайсинга гена *PLD1*: c.2430-1G>A, ранее не описанная однонуклеотидная делеция гена *SIDT1*: c.2426del (p.Leu809CysfsTer2), новый миссенс-вариант c.10313C>G (p.Pro3438Arg) гена *LRP1B* и вариант однонуклеотидной делеции rs753876598: c.165del (p.Ser56AlafsTer11) гена *CETP*. Все варианты впервые описаны у пробандов с клиническим диагнозом «семейная гиперхолестеринемия». Идентифицированы варианты, которые потенциально могут влиять на формирование фенотипа семейной гиперхолестеринемии.
Ключевые слова: семейная гиперхолестеринемия; полногеномное секвенирование; машинное обучение; *SIDT1*; *LRP1B*; *PLD1*; *CETP*.

Introduction

One of the most common congenital metabolic disorders is familial hypercholesterolemia (FH) (Ezhov et al., 2019). Familial hypercholesterolemia is a condition caused by a type of genetic defects leading to a decreased rate of removal of low-density lipoproteins from the bloodstream and a pronounced increase in the blood level of total cholesterol (Ezhov et al., 2019). This illness leads to early development of cardiovascular diseases of atherosclerotic origin (Wiegman et al., 2015; Santos et al., 2016; Borén et al., 2020). Familial hypercholesterolemia is a monogenic disease with predominantly autosomal dominant inheritance (Ezhov et al., 2019).

The prevalence of the heterozygous type of FH in white populations is 1 per 250 people (Ezhov et al., 2019). Rare pathogenic variants in the *LDLR* gene are present in 75–85 % of cases with an identified molecular genetic cause of the disease, and variants in other genes (*APOB*, *PCSK9*, *LDLRAP1*, *ABCG5*, *ABCG8*, and others) occur in this group of patients with a frequency of less than 5 % (Nordestgaard et al., 2013; Iacocca, Hegele, 2017; Vasilyev et al., 2020). Patients can be homozygous or heterozygous carriers of pathogenic variants, and this status determines the severity of the disease and onset age of manifestations of cardiovascular complications (Vaezi, Amini, 2022). A negative result of genetic screening for pathogenic variants does not rule out familial hypercholesterolemia. In 20–40 % of cases, changes in the above genes are absent according to molecular genetic analysis. The risk of coronary heart disease among patients with FH is 20 times higher in the absence of treatment (Khera et al., 2016); therefore, it is important and relevant to search for new approaches to identifying patients at early disease stages and to assessing predisposition to this disease in patients' families.

To search for new cases, some authors have proposed a classifier to identify potential patients with FH by means of electronic medical records. Using data from patients with confirmed FH (n = 197) and cases without FH (n = 6590), a decision tree classifier was trained in that study. The classifier showed a positive predictive value (PPV) of 0.88 and a sensitivity of 0.75 for long-term testing. This classifier proved to be effective at finding candidate patients for further screening for familial hypercholesterolemia. Such machine-learning-based strategies can result in efficient identification of patients having the highest risk of the disease (Banda et al., 2019).

For the diagnosis of FH, clinicians use the principle of cascade genetic screening. The latter is a step-by-step identification of patients with familial hypercholesterolemia. When elevated blood levels of total cholesterol and low-density lipoprotein cholesterol (LDL-C) are detected in a patient, his/her family history of health problems is collected and clinical manifestations are analyzed. In case of a diagnosis of "probable" or "definite" FH according to the Dutch Lipid Clinic Network Criteria (Geneva: World Health Organization), the patient is referred for molecular genetic testing. The cascade screening includes quantitation of blood lipids in all first-degree relatives of the proband. If the FH diagnosis is confirmed by the molecular genetic methods, then genetic screening is performed on his/her relatives. As new patients with FH are identified, their relatives are examined too. The cascade screening is the most effective way to detect previously undiagnosed FH (Nordestgaard et al., 2013; Ezhov et al., 2019).

The aim of the present work was to search for new genes associated with the FH phenotype using modern high-tech methods of sequencing and machine learning.

Materials and methods

A group of patients with FH (ICD-10 E78.0, E78.2, n = 102), was recruited from a clinical diagnostic department at the Institute of Internal and Preventive Medicine (IIPM) – a branch of the Institute of Cytology and Genetics, the Siberian Branch of the Russian Academy of Sciences (ICG SB RAS). The study protocol was approved by the Ethics Committee at the IIPM – a branch of the ICG SB RAS, decision No. 68 of June 4, 2019. Informed consent was obtained from each study participant.

The diagnosis of FH was made in accordance with the clinical lipid criteria of the Dutch Lipid Clinic Network (DLCN) (WHO-Human genetics DoNDP…, 1999). According to the criteria, a score was computed (see the Supplementary)[1] for patients with familial hypercholesterolemia. The patients underwent a medical examination, ultrasonographic diagnostics, and blood sampling for biochemical assays (lipid profiling and general analysis of biochemical parameters) and molecular genetic assays.

Blood samples for the biochemical analyses were collected once from the cubital vein in the morning on an empty stomach at 12 h after a meal. Serum levels of total cholesterol, triglycerides, LDL-C, high-density lipoprotein cholesterol (HDL-C), and glucose were determined by enzymatic methods on a KoneLab300i automatic biochemical analyzer (Finland) with reagents from TermoFisher (Finland). The LDL-C level was calculated via the Friedewald formula; at the LDL-C concentration of > 4.5 mmol/L, the method of direct quantification of LDL-C was used. Statistical analysis of the data was performed in the SPSS software for Windows, version 23.0.

Phenol-chloroform extraction was carried out to isolate DNA from blood (Sambrook, Russell, 2006). The quality of the extracted DNA was assessed with the help of an Agilent 2100 Bioanalyzer capillary electrophoresis system (Agilent Technologies Inc., USA).

Targeted DNA sequencing in patients with FH was performed on the MiSeq platform (Illumina) using a custom-designed panel of 43 genes: *LDLR*, *APOB*, *PCSK9*, *LDLRAP1*, *CETP*, *LPL*, *HMGCR*, *NPC1L1*, *PPARA*, *MTTP*, *LMF1*, *SAR1B*, *ABCA1*, *ABCG5*, *ABCG8*, *CYP7A1*, *STAP1*, *LIPA*, *PNPLA5*, *APOA1*, *APOA5*, *APOC2*, *APOE*, *LCAT*, *ANGPTL3*, *LIPC*, *APOA4*, *APOC3*, *SREBF1*, *LMNA*, *PPARG*, *PLIN1*, *POLD1*, *LPA*, *SMAD1*, *SMAD2*, *SMAD3*, *SMAD4*, *SMAD5*, *SMAD6*, *SMAD7*, *SMAD9*, and *LIPG* (NimbleGen SeqCap Target Enrichment, Roche, Switzerland).

At the next stage of this work, from the study population, 42 patients with FH were chosen who did not show pathogenic variants in the tested genes during the targeted sequencing analysis. These patients were subjected to multiplex ligation-dependent probe amplification (MLPA) analysis to identify possible sequence alterations (deletions or duplications) in the *LDLR* gene promoter and exons by means of SALSA MLPA KIT P062 (MRCHolland, Amsterdam, the Netherlands).

Using the group of patients with FH (compiled according to the DLCN criteria and including cases of the disease confirmed by molecular genetic analysis), decision trees were constructed, which enabled us to identify cases in this group that require additional molecular genetic analysis. Software was written in Python 3.9 for building a set of decision rules for predicting FH on the basis of machine learning with a limited training set. The decision rules were stored as data representation in the Predictive Model Markup Language. The decision rules were built by means of a labeled database of patients with a diagnosis of FH (Certificate of Database Registration: RU No. 2023660511; software registration application of May 2, 2023).

By machine learning methods, five probands with the severest FH without pathogenic variants in the tested genes were identified for subsequent whole-genome sequencing on the HiSeq 1500 platform (Illumina). Automated processing and annotation of the obtained sequencing data were conducted on the NGS Wizard platform (genomenal.ru). The sequence reads were mapped to the reference human genome (GRCh38/hg38). A potential effect of novel missense variants on protein function/structure was assessed using data from *in silico* prediction tools (CADD (https://cadd.gs.washington.edu/snv), PolyPhen2 (http://genetics.bwh.harvard.edu/pph2/), and MutationTaster (https://www.mutationtaster.org/)) and data from gnomAD on the frequency of these variants in populations. In this way, variants (in genes associated with lipid metabolism) leading to a loss of protein function and missense variants with a frequency of less than 0.01 % were selected. Pathogenicity of new variants was evaluated according to the guidelines of the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (Richards et al., 2015). Analysis of protein-protein interaction networks was performed in STRING (Szklarczyk et al., 2019).

Results

By targeted sequencing and MLPA, "pathogenic" and "likely pathogenic" variants were detected in 47.5 % of the examined probands and in 85.7 % of the probands' children. Variants in the *LDLR* gene that were identified in patients with the FH phenotype in our study are presented in Table 1. All missense variants were found to be in a heterozygous state. Variants Cys352Tyr, Cys340Phe, and Leu401His have been previously described in patients with FH in Russia (Zakharova et al., 2005) and in other countries (Feussner et al., 1996; Torres et al., 2014).

In the study population, the heterozygous type of the disease was due to rare variants in the *LDLR* gene in 73 % of the cases. Two new variants – NM_000527.5:c.266G>C, NP_000518.1:p.Cys89Ser and NM_000527.5:c.1123T>G, NP_000518.1:p.Tyr375Asp – were identified in the *LDLR* gene. Two unrelated probands turned out to be carriers of compound heterozygous variants of the *LDLR* gene, whereas the clinical course of the disease in these patients corresponded to the homozygous type of familial hypercholesterolemia. In the first case, in a 28-year-old female patient with a diagnosis of definite FH, we detected rare variants NM_000527.5:c.796G>A, NP_000518.1:p.Asp266Asn, and NM_000527.5:c.1054T>A, NP_000518.1:p.Cys352Ser in exons 5 and 7 of *LDLR* (see Table 1). In the second case, a 35-year-old female patient with a diagnosis of definite FH has two missense variants in exons 3 and 8 of *LDLR*. One substitution is located in *LDLR* exon 3 (NM_000527.5:c.266G>C, NP_000518.1:p.Cys89Ser), in which at this position, rare likely pathogenic variant rs875989894 NM_000527.5:c.266G>A, NP_000518.1:p.Cys89Tyr has been previously described in patients with FH (Day et al., 1997; Graham et al., 1999; Fouchier et al., 2005).

The pathogenicity of the identified variant was confirmed by *in silico* analysis (Mutation Taster score: 112, CADD score: 24.8, PolyPhen-2 score: 1.000). The other new missense variant is situated in exon 8 of *LDLR*: NM_000527.5:c.1123T>G, NP_000518.1:p.Tyr375Asp (see Table 1). This missense variant causes an amino acid substitution at the same position where other missense variants have been described before as

[1] Supplementary Material is available in the online version of the paper: http://vavilov.elpub.ru/jour/manager/files/Suppl_Ivanoshchuk_Engl_27_5.pdf

Table 1. Single-nucleotide variants in genes *LDLR*, *APOB*, and *LPL* of patients with the FH phenotype

Variant ID	Amino acid substitution	Minor allele frequency, gnomAD (v2.1.1)	Interpretation of the nucleotide sequence variant, according to database ClinVar or LOVD
		LDLR	
rs121908038	p.Leu401His	ND	Likely pathogenic
rs137853964	p.Val827Ile	A = 0.000919	
rs28942078	p.Val429Met	A = 0.000012	Pathogenic
rs539080792	p.Glu337Lys	A = 0.000106	
rs570942190	p.Arg416Trp	T = 0.000024	
rs755757866	p.Cys340Tyr	T = 0.000008	Likely pathogenic
rs761954844	p.Cys329Tyr	A = 0.000025	
rs879254566	p.Asp178Glu	ND	Pathogenic
rs879254721	p.Glu308Lys	ND	
rs879254980	p.Glu558Ter	ND	
rs879255191	–	ND	Likely pathogenic
rs875989907	p.Asp266Asn	A = 0.000012	Pathogenic
rs879254769	p.Cys352Ser	ND	Likely pathogenic
ND/rs875989894	p.Cys89Ser	ND	New variant
ND	p.Tyr375Asp	ND	
		APOB	
rs5742904	p.Arg3527Gln	T = 0.000294	Pathogenic
		LPL	
rs118204077	p.Arg270Cys	C = 0.000008	Pathogenic

Note. ND – no data; GenBank accession numbers of protein sequences that were used in variant annotation: *LDLR* (NP_000518.1), *APOB* (NP_000375.3), and *LPL* (NP_000228.1).

likely pathogenic in patients with FH (Assouline et al., 1997; García-García et al., 2001; Damgaard et al., 2005; Mollaki et al., 2014). The pathogenicity of the variant was also confirmed by *in silico* analysis (Mutation Taster score: 160, CADD score: 25.5, PolyPhen-2 score: 1.000). Both detected variants (NM_000527.5:c.266G>C, NP_000518.1:p.Cys89Ser and NM_000527.5:c.1123T>G, NP_000518.1:p.Tyr375Asp) are not annotated in the gnomAD database (v2.1.1). According to this evidence, both were assumed to be likely pathogenic variants.

Patients without functionally significant substitutions in lipid metabolism genes were analyzed by MLPA to determine sequence changes (deletions or duplications) in the promoter and exons of the *LDLR* gene. This assay revealed a deletion of a coding region in the *LDLR* gene [NM_000527.4:c.(2140+1_2141-1)_(2311+1_2312-1)del] in DNA samples from two unrelated patients.

In the molecular genetic analysis, in three patients from two unrelated families (a proband and a son of the proband from one family and a proband from another family), we identified variant rs5742904 (NM_000384.3:c.10580G>A, NP_000375.3:p.Arg3527Gln) (ClinVar Variation ID:17890) in the *APOB* gene (see Table 1).

Rare substitutions in the *APOB* gene region encoding the LDL receptor-inding site are associated with hypercholesterolemia. One of the variants in this region, NP_000375.3:p.Arg3527Gln, leads to hypercholesterolemia with reduced clearance of LDL-C owing to a defect in the structural motif of LDL that is responsible for affinity for LDL receptor (Pullinger et al., 1995).

Analysis of our data of targeted high-throughput sequencing revealed rare pathogenic variant rs118204077 (NM_000237.3:c.808C>T, NP_000228.1:p.Arg270Cys) in the *LPL* gene in a heterozygous state (ClinVar Variation ID: 1548) (see Table 1). This variant was found in a 45-year-old male patient with hypercholesterolemia (12.4 mmol/L) and hypertriglyceridemia (17.4 mmol/L; DLCN score: 5). Earlier, variants associated with hypertriglyceridemia have been described at this locus (Ma et al., 1994; Surendran et al., 2012) in patients with lipoprotein lipase deficiency (Hegele et al., 2018; Teramoto et al., 2018).

According to the results of the molecular genetic analyses, 52.5 % of our patients are not carriers of pathogenic variants in the studied lipid metabolism genes. Among these patients, by a machine learning algorithm, five subjects with the severest FH were chosen for whole-genome sequencing. As a result, in three patients, four variants with a minor allele frequency of less than 0.01 % were identified in genes related to lipid metabolism. Among these variants, two are single-nucleotide deletions, one affects a splicing acceptor site, and one is a missense variant. The findings are presented in Table 2.

In the *SIDT1* gene (encoding a protein called SID1 transmembrane family member 1), a new previously undescribed variant was detected that yields a frameshift starting with

Table 2. Rare variants identified in lipid metabolism genes by whole-genome sequencing in patients with the FH phenotype

Gene	Nucleotide sequence position (GRCh38/hg38)	Position in cDNA (transcript's GenBank accession No.)	Amino acid substitution (protein's GenBank accession No.)	Genotype	Minor allele frequency (gnomAD v2.1.1)	Minor allele frequency (RuSeq)	dbSNP ID
SIDT1	chr3:113627646 GT>G	(NM_017699.3) c.2426del	(NP_060169.2) p.Leu809CysfsTer2	Heterozygote	–	–	New
LRP1B	chr2:140442605 G>C	(NM_018557.3) c.10313C>G	(NP_061027.2) p.Pro3438Arg	Heterozygote	–	–	New
PLD1	chr3:171645024 G>A	(NM_002662.5) c.2430, –1G>A	–	Heterozygote	0.00001768	0.0002457	rs760657350
CETP	chr16:56963054 GC>G	(NM_000078.3) c.165del	(NP_000069.2) p.Ser56AlafsTer11	Heterozygote	0.00001415	0.0002081	rs753876598

codon 809 (NM_017699.3:c.2426de, NP_060169.2:p.Leu809CysfsTer2). The gene consists of 30 exons and is located in chromosomal region 3q13.2 (https://www.ncbi.nlm.nih.gov/gene/54847). According to gnomAD, loss-of-function variants in this gene have been documented, but according to ClinVar and the literature, none of them have been annotated as pathogenic or likely pathogenic. For this gene, the pLI score was found to be 0, indicating that the gene is resistant to loss-of-function variants. The weight of evidence suggested that this variant has uncertain clinical significance (pathogenicity criterion PM2).

A new missense variant was found in the *LRP1B* gene (LDL receptor-related protein 1B): c.10313C>G p.Pro3438Arg in a heterozygous state. The gene is located in chromosomal region 2q22.1-q22.2, consists of 92 exons, and encodes one of the receptors of the LDL receptor family (https://www.ncbi.nlm.nih.gov/gene/53353). In gnomAD, there are no data on the frequency of this variant. The pathogenicity of this variant was also corroborated by *in silico* analysis (Mutation Taster score: 103, CADD score: 33, PolyPhen-2 score: 1.000). Most variants in this gene either are benign (data from ClinVar, accessed in February 2023) or have uncertain clinical significance. The totality of the data indicated that this variant has uncertain clinical significance (pathogenicity criteria PM2, PP3, and BP1).

One of our patients proved to be a carrier of a rare heterozygous variant at a splicing acceptor site (NM_002662.5:c.2430, –1G>A) in the phospholipase D1 (*PLD1*) gene. This variant is registered in a control sample in gnomAD: five mutant alleles on 282,768 chromosomes (no homozygotes have been detected). The *PLD1* gene codes for a phosphatidylcholine-specific phospholipase that catalyzes the hydrolysis of phosphatidylcholine, thus yielding phosphatidic acid and choline (https://www.ncbi.nlm.nih.gov/gene/5337). The gene is situated in chromosomal region 3q26.31 and contains 35 exons. Phospholipase D (PLD) and its enzymatic reaction product, phosphatidic acid, regulate adhesion of immune cells (macrophages and neutrophils) to collagen (Speranza et al., 2014).

It is known that biallelic variants with loss of function of the *PLD1* gene cause neonatal cardiomyopathy and congenital malformations of the pulmonary valve and tricuspid valve, of the right ventricle of the heart, and of the outflow tract of the right ventricle (Ta-Shma et al., 2017; Lahrouchi et al., 2021). The weight of evidence suggested that this substitution is a likely pathogenic variant in relation to congenital heart malformations (pathogenicity criteria PM2 and PVS1). With respect to FH, we categorized the detected substitution as a variant of unknown clinical significance (pathogenicity criterion PM2).

One of the examined patients was found to have a heterozygous single-nucleotide deletion in *CETP*: rs753876598 (NM_000078.3:c.165del) (https://www.ncbi.nlm.nih.gov/gene/1071). The variant is annotated in the ClinVar database (ID1675625) and is registered in a control sample of gnomAD: four mutant alleles on 282,774 chromosomes (no homozygotes have been found). It is known that variants causing loss of function of this gene affect the HDL-C level (Millwood et al., 2018; Li et al., 2021). According to the totality of criteria for pathogenicity evaluation (PM2 and PVS1), we designated this variant as likely pathogenic. The *CETP* gene codes for a plasma protein that catalyzes the exchange of triglycerides and cholesterol esters between lipoprotein particles (Oliveira, Raposo, 2020).

Discussion

High-throughput sequencing is employed not only for molecular genetic diagnosis of FH but also as a tool for identifying i) variants that may be involved in lipid metabolism and ii) their effects on the phenotype of patients with FH (Miroshnikova et al., 2021). In the current study, 16 variants were identified in an FH population (15 single-nucleotide substitutions and one deletion) that have previously been classified as pathogenic or likely pathogenic in the ClinVar or LOVD database as well as two new missense variants in the *LDLR* gene that we classified as pathogenic. In our genome-wide analysis, in lipid metabolism-associated genes, we detected four additional variants that met our search criteria. Two of these four variants have been described before, and two are new.

One of the genes in which rare variants were found in patients with FH is *PLD1*, encoding phospholipase D1. This enzyme hydrolyzes membrane lipid phosphatidylcholine thereby generating phosphatidic acid (Bowling et al., 2021). Phosphatidic acid is an intermediate metabolite in the synthesis of all membrane glycerophospholipids and plays an important structural role in live cells by promoting membrane

biogenesis (Tanguy et al., 2018); furthermore, its involvement and phospholipase D1's participation in exocytosis have been demonstrated (Tanguy et al., 2022).

Alternative splicing of *PLD1* mRNA results in many different transcripts having both catalytic and regulatory functions (Nelson, Frohman, 2015). It has been shown that recessive variants in the *PLD1* gene are associated with severe right-sided congenital heart malformations in two families (Ta-Shma et al., 2017). In *Pld1* knockout mice, moderate dysfunction of pulmonary and tricuspid valves is observed (Ta-Shma et al., 2017). Recessive *PLD1* variants also correlate with isolated neonatal cardiomyopathies (Lahrouchi et al., 2021). In humans, missense variants of *PLD1* are reported to be concentrated in regions of the protein critical for catalytic activity, thus resulting in low enzymatic activity in most of such mutant proteins (Lahrouchi et al., 2021). It has also been demonstrated in cell lines that *PLD1* overexpression promotes the formation of lipid droplets, whereas an siRNA *PLD1* knockdown inhibits this process (Andersson et al., 2006).

The variant (NM_002662.5:c.2430, –1G>A) that we detected in a proband with FH without signs of congenital heart disease is in a heterozygous state. Considering the low prevalence of this variant and its possible role in subcellular transport and in the formation of lipid droplets, this substitution is of interest for further investigation in individuals with lipid metabolism disorders.

Another rare variant was found by us in the *LRP1B* gene (LDL receptor-related protein 1B). The LRP1B protein is a member of the LDL receptor family (Strickland et al., 2002). LRP1B takes part in lipoprotein catabolism; accordingly, research on rare variants of the *LRP1B* gene in individuals with FH is promising. Most of the recently identified ligands of LRP1B are well-known factors of blood coagulation and of lipoprotein metabolism, suggesting that LRP1B is implicated in atherosclerosis (Lee, 2019).

SIDT1 is a multispan transmembrane protein belonging to the SID1 transmembrane family and shares some sequence homology with *Caenorhabditis elegans* ChUP-1, which is a cholesterol-binding protein located in intracellular vesicles (Valdes et al., 2012). SIDT1 expression in endolysosomes has been documented (Nguyen et al., 2019). *SIDT1* has been shown to participate in cholesterol transport (Méndez-Acevedo et al., 2017) but has not been investigated in the context of the FH phenotype. Most likely, the variant that we found in this gene does not take part in the formation of the clinical phenotype of FH because our assessment using the American College of Medical Genetics and Genomics criteria classifies it as a variant of uncertain clinical significance; however, for unambiguous evaluation of its association with the FH phenotype, additional data are needed.

The *CETP* gene codes for the CETP protein, which carries cholesterol esters. This protein regulates the concentration and particle size of HDL-C in the blood and plays an important role in reverse cholesterol transport (Barter, Kastelein, 2006). It has been shown that elevated activity of *CETP* reduces HDL-C concentration and correlates with a higher risk of cardiovascular disease (Barter, 2011; Iwanicka et al., 2018). Variants in the *CETP* gene can alter the blood lipid profile (Wuni et al., 2022). In our previous study on one of the *CETP* variants, we reported its association with changes in the blood lipid profile and with the risk of myocardial infarction in a population of Western Siberia (Semaev et al., 2019). When a map of functional and physical associations was constructed in the present study, the APOB protein turned out to be a predicted functional partner of the CETP protein, and mutations in APOB represent some of FH etiologies.

Additional segregational and functional analyses are necessary to evaluate pathogenic effects of the identified variants on the formation of the clinical FH phenotype. Identification of new pathogenic variants will facilitate risk assessment of FH and of its complications among patients and members of their families.

Conclusion

A combination of machine learning and whole-genome sequencing in probands with a clinical diagnosis of FH revealed rare variants in genes *SIDT1*, *LRP1B*, *PLD1*, and *CETP*; these variants may influence the disease phenotype.

References

Andersson L., Boström P., Ericson J., Rutberg M., Magnusson B., Marchesan D., Ruiz M., Asp L., Huang P., Frohman M.A., Borén J., Olofsson S.O. PLD1 and ERK2 regulate cytosolic lipid droplet formation. *J. Cell Sci*. 2006;119(Pt. 11):2246-2257. DOI 10.1242/jcs.02941.

Assouline L., Leitersdorf E., Lambert M., Reshef A., Feoli-Fonseca J.C., Levy E. Identification of two novel LDL receptor gene defects in French-Canadian pediatric population: mutational analysis and biochemical studies. *Hum. Mutat*. 1997;9(6):555-562. DOI 10.1002/(SICI)1098-1004(1997)9:6<555::AID-HUMU9>3.0.CO;2-0.

Banda J.M., Sarraju A., Abbasi F., Parizo J., Pariani M., Ison H., Briskin E., Wand H., Dubois S., Jung K., Myers S.A., Rader D.J., Leader J.B., Murray M.F., Myers K.D., Wilemon K., Shah N.H., Knowles J.W. Finding missed cases of familial hypercholesterolemia in health systems using machine learning. *NPJ Digit. Med*. 2019;2:23. DOI 10.1038/s41746-019-0101-5.

Barter P.J. The causes and consequences of low levels of high density lipoproteins in patients with diabetes. *Diabetes Metab. J*. 2011;35(2):101-106. DOI 10.4093/dmj.2011.35.2.101.

Barter P.J., Kastelein J.J.P. Targeting cholesteryl ester transfer protein for the prevention and management of cardiovascular disease. *J. Am. Coll. Cardiol*. 2006;47(3):492-499. DOI 10.1016/j.jacc.2005.09.042.

Borén J., Chapman M.J., Krauss R.M., Packard C.J., Bentzon J.F., Binder C.J., Daemen M.J., Demer L.L., Hegele R.A., Nicholls S.J., Nordestgaard B.G., Watts G.F., Bruckert E., Fazio S., Ference B.A., Graham I., Horton J.D., Landmesser U., Laufs U., Masana L., Pasterkamp G., Raal F.J., Ray K.K., Schunkert H., Taskinen M.R., van de Sluis B., Wiklund O., Tokgozoglu L., Catapano A.L., Ginsberg H.N. Low-density lipoproteins cause atherosclerotic cardiovascular disease: pathophysiological, genetic, and therapeutic insights: a consensus statement from the European Atherosclerosis Society Consensus Panel. *Eur. Heart J*. 2020;41(24):2313-2330. DOI 10.1093/eurheartj/ehz962.

Bowling F.Z., Frohman M.A., Airola M.V. Structure and regulation of human phospholipase D. *Adv. Biol. Regul.* 2021;79:100783. DOI 10.1016/j.jbior.2020.100783.

Damgaard D., Larsen M.L., Nissen P.H., Jensen J.M., Jensen H.K., Soerensen V.R., Jensen L.G., Faergeman O. The relationship of molecular genetic to clinical diagnosis of familial hypercholesterolemia in a Danish population. *Atherosclerosis*. 2005;180(1):155-160. DOI 10.1016/j.atherosclerosis.2004.12.001.

Day I.N., Whittall R.A., O'Dell S.D., Haddad L., Bolla M.K., Gudnason V., Humphries S.E. Spectrum of LDL receptor gene mutations in heterozygous familial hypercholesterolemia. *Hum. Mutat*.

1997;10(2):116-127. DOI 10.1002/(SICI)1098-1004(1997)10:2<116::AID-HUMU4>3.0.CO;2-I.

Ezhov M.V., Bazhan S.S., Ershova A.I., Meshkov A.N., Sokolov A.A., Kukharchuk V.V., Gurevich V.S., Voevoda M.I., Sergienko I.V., Shakhtshneider E.V., Pokrovsky S.N., Konovalov G.A., Leontyeva I.V., Konstantinov V.O., Shcherbakova M.Yu., Zakharova I.N., Balakhonova T.V., Filippov A.E., Akhmedzhanov N.M., Aleksandrova O.Yu., Lipovetsky B.M. Clinical guidelines for familial hypercholesterolemia. *Ateroscleroz i Dislipidemii = The Journal of Atherosclerosis and Dyslipidemias.* 2019;15(1):58-98. (in Russian)

Feussner G., Dobmeyer J., Nissen H., Hansen T.S. Unusual xanthomas in a young patient with heterozygous familial hypercholesterolemia and type III hyperlipoproteinemia. *Am. J. Med. Genet*. 1996; 65:149-154. DOI 10.1002/(SICI)1096-8628(19961016)65:2<149::AID-AJMG14>3.0.CO;2-Q.

Fouchier S.W., Kastelein J.J., Defesche J.C. Update of the molecular basis of familial hypercholesterolemia in the Netherlands. *Hum. Mutat*. 2005;26(6):550-556. DOI 10.1002/humu.20256.

García-García A.B., Real J.T., Puig O., Cebolla E., Marín-García P., Martínez Ferrandis J.I., García-Sogo M., Civera M., Ascaso J.F., Carmena R., Armengod M.E., Chaves F.J. Molecular genetics of familial hypercholesterolemia in Spain: Ten novel *LDLR* mutations and population analysis. *Hum. Mutat*. 2001;18(5):458-459. DOI 10.1002/humu.1218.

Graham C.A., McClean E., Ward A.J., Beattie E.D., Martin S., O'Kane M., Young I.S., Nicholls D.P. Mutation screening and genotype:phenotype correlation in familial hypercholesterolaemia. *Atherosclerosis*. 1999;147(2):309-316. DOI 10.1016/s0021-9150(99)00201-4.

Hegele R.A., Berberich A.J., Ban M.R., Wang J., Digenio A., Alexander V.J., D'Erasmo L., Arca M., Jones A., Bruckert E., Stroes E.S., Bergeron J., Civeira F., Witztum J.L., Gaudet D. Clinical and biochemical features of different molecular etiologies of familial chylomicronemia. *J. Clin. Lipidol*. 2018;12(4):920-927.e4. DOI 10.1016/j.jacl.2018.03.093.

Iacocca M.A., Hegele R.A. Recent advances in genetic testing for familial hypercholesterolemia. *Expert. Rev. Mol. Diagn*. 2017;17(7): 641-651. DOI 10.1080/14737159.2017.1332997.

Iwanicka J., Iwanicki T., Niemiec P., Balcerzyk A., Krauze J., Górczyńska-Kosiorz S., Ochalska-Tyka A., Grzeszczak W., Żak I. Relationship between *CETP* gene polymorphisms with coronary artery disease in Polish population. *Mol. Biol. Rep*. 2018;45(6):1929-1935. DOI 10.1007/s11033-018-4342-1.

Khera A.V., Won H.H., Peloso G.M., Lawson K.S., Bartz T.M., Deng X., van Leeuwen E.M., … Correa A., Boerwinkle E., Merlini P.A., Ardissino D., Saleheen D., Gabriel S., Kathiresan S. Diagnostic yield and clinical utility of sequencing familial hypercholesterolemia genes in patients with severe hypercholesterolemia. *J. Am. Coll. Cardiol*. 2016;67(22):2578-2589. DOI 10.1016/j.jacc.2016.03.520.

Lahrouchi N., Postma A.V., Salazar C.M., De Laughter D.M., Tjong F., Piherová L., Bowling F.Z., … Mulder B., Airola M.V., Kmoch S., Barnett J.V., Clur S.A., Frohman M.A., Bezzina C.R. Biallelic loss-of-function variants in *PLD1* cause congenital right-sided cardiac valve defects and neonatal cardiomyopathy. *J. Clin. Invest*. 2021; 131(5):e142148. DOI 10.1172/JCI142148.

Lee S. The genetic and epigenetic association of LDL Receptor Related Protein 1B (*LRP1B*) gene with childhood obesity. *Sci. Rep*. 2019; 9(1):1815. DOI 10.1038/s41598-019-38538-2.

Li W., Liu X., Huang C., Liu L., Tan X., Wang X. The loss-of-function mutation of *CETP* affects HDLc levels but not ApoA1 in patients with acute myocardial infarction. *Nutr. Metab. Cardiovasc. Dis*. 2021;31(2):602-607. DOI 10.1016/j.numecd.2020.10.019.

Ma Y., Liu M.S., Chitayat D., Bruin T., Beisiegel U., Benlian P., Foubert L., De Gennes J.L., Funke H., Forsythe I., Blaichman S., Papanikolaou M., Erkelens D.W., Kastelein J., Brunzell J.D., Hayden M.R. Recurrent missense mutations at the first and second base of codon Arg^{243} in human lipoprotein lipase in patients of different ancestries. *Hum. Mutat*. 1994;3(1):52-58. DOI 10.1002/humu.1380030109.

Méndez-Acevedo K.M., Valdes V.J., Asanov A., Vaca L. A novel family of mammalian transmembrane proteins involved in cholesterol transport. *Sci. Rep*. 2017;7(1):7450. DOI 10.1038/s41598-017-07077-z.

Millwood I.Y., Bennett D.A., Holmes M.V., Boxall R., Guo Y., Bian Z., Yang L., Sansome S., Chen Y., Du H., Yu C., Hacker A., Reilly D.F., Tan Y., Hill M.R., Chen J., Peto R., Shen H., Collins R., Clarke R., Li L., Walters R.G., Chen Z., China Kadoorie Biobank Collaborative Group. Association of *CETP* gene variants with risk for vascular and nonvascular diseases among chinese adults. *JAMA Cardiol*. 2018;3(1):34-43. DOI 10.1001/jamacardio.2017.4177.

Miroshnikova V.V., Romanova O.V., Ivanova O.N., Fedyakov M.A., Panteleeva A.A., Barbitoff Y.A., Muzalevskaya M.V., Urazgildeeva S.A., Gurevich V.S., Urazov S.P., Scherbak S.G., Sarana A.M., Semenova N.A., Anisimova I.V., Guseva D.M., Pchelina S.N., Glotov A.S., Zakharova E.Y., Glotov O.S. Identification of novel variants in the *LDLR* gene in Russian patients with familial hypercholesterolemia using targeted sequencing. *Biomed. Rep*. 2021;14(1):15. DOI 10.3892/br.2020.1391.

Mollaki V., Progias P., Drogari E. Familial hypercholesterolemia in Greek children and their families: genotype-to-phenotype correlations and a reconsideration of *LDLR* mutation spectrum. *Atherosclerosis*. 2014;237(2):798-804. DOI 10.1016/j.atherosclerosis.2014.09.031.

Nelson R.K., Frohman M.A. Physiological and pathophysiological roles for phospholipase D. *J. Lipid. Res*. 2015;56(12):2229-2237. DOI 10.1194/jlr.R059220.

Nguyen T.A., Smith B.R.C., Elgass K.D., Creed S.J., Cheung S., Tate M.D., Belz G.T., Wicks I.P., Masters S.L., Pang K.C. SIDT1 localizes to endolysosomes and mediates double-stranded RNA transport into the cytoplasm. *J. Immunol*. 2019;202(12):3483-3492. DOI 10.4049/jimmunol.1801369.

Nordestgaard B.G., Chapman M.J., Humphries S.E., Ginsberg H.N., Masana L., Descamps O.S., Wiklund O., … Kuivenhoven J.A., Pajukanta P., Ray K., Stalenhoef A.F., Stroes E., Taskinen M.R., Tybjærg-Hansen A., European Atherosclerosis Society Consensus Panel. Familial hypercholesterolaemia is underdiagnosed and undertreated in the general population: guidance for clinicians to prevent coronary heart disease: consensus statement of the European Atherosclerosis Society. *Eur. Heart J*. 2013;34(45):3478-3490. DOI 10.1093/eurheartj/eht273.

Oliveira H.C.F., Raposo H.F. Cholesteryl ester transfer protein and lipid metabolism and cardiovascular diseases. In: Jiang X.-C. (Ed.). Lipid Transfer in Lipoprotein Metabolism and Cardiovascular Disease. Advances in Experimental Medicine and Biology. Vol. 1276. Singapore: Springer, 2020;15-25. DOI 10.1007/978-981-15-6082-8_2.

Pullinger C.R., Hennessy L.K., Chatterton J.E., Liu W., Love J.A., Mendel C.M., Frost P.H., Malloy M.J., Schumaker V.N., Kane J.P. Familial ligand-defective apolipoprotein B. Identification of a new mutation that decreases LDL receptor binding affinity. *J. Clin. Invest*. 1995;95(3):1225-1234. DOI 10.1172/JCI117772.

Richards S., Aziz N., Bale S., Bick D., Das S., Gastier-Foster J., Grody W.W., Hegde M., Lyon E., Spector E., Voelkerding K., Rehm H.L., 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(5):405-424. DOI 10.1038/gim.2015.30.

Sambrook J., Russell D.W. Purification of nucleic acids by extraction with phenol:chloroform. *Cold Spring Harb. Protoc*. 2006;2006(1): pdb.prot4455. DOI 10.1101/pdb.prot4455.

Santos R.D., Gidding S.S., Hegele R.A., Cuchel M.A., Barter P.J., Watts G.F., Baum S.J., Catapano A.L., Chapman M.J., Defesche J.C., Folco E., Freiberger T., Genest J., Hovingh G.K., Harada-Shiba M., Humphries S.E., Jackson A.S., Mata P., Moriarty P.M., Raal F.J., Al-Rasadi K., Ray K.K., Reiner Z., Sijbrands E.J., Yamashita S., International Atherosclerosis Society Severe Familial Hypercholesterolemia Panel. Defining severe familial hypercholesterolaemia and the

implications for clinical management: a consensus statement from the International Atherosclerosis Society Severe Familial Hypercholesterolemia Panel. *Lancet Diabetes Endocrinol*. 2016;4(10):850-861. DOI 10.1016/S2213-8587(16)30041-9.

Semaev S., Shakhtshneider E., Orlov P., Ivanoshchuk D., Malyutina S., Gafarov V., Ragino Y., Voevoda M. Association of rs708272 (*CETP* gene variant) with lipid profile parameters and the risk of myocardial infarction in the white population of Western Siberia. *Biomolecules*. 2019;9(11):739. DOI 10.3390/biom9110739.

Speranza F., Mahankali M., Henkels K.M., Gomez-Cambronero J. The molecular basis of leukocyte adhesion involving phosphatidic acid and phospholipase D. *J. Biol. Chem*. 2014;289(42):28885-28897. DOI 10.1074/jbc.M114.597146.

Strickland D.K., Gonias S.L., Argraves W.S. Diverse roles for the LDL receptor family. *Trends Endocrinol. Metab*. 2002;13(2):66-74. DOI 10.1016/s1043-2760(01)00526-4.

Surendran R.P., Visser M.E., Heemelaar S., Wang J., Peter J., Defesche J.C., Kuivenhoven J.A., Hosseini M., Péterfy M., Kastelein J.J., Johansen C.T., Hegele R.A., Stroes E.S., Dallinga-Thie G.M. Mutations in *LPL*, *APOC2*, *APOA5*, *GPIHBP1* and *LMF1* in patients with severe hypertriglyceridaemia. *J. Intern. Med*. 2012;272(2):185-196. DOI 10.1111/j.1365-2796.2012.02516.x.

Szklarczyk D., Gable A.L., Lyon D., Junge A., Wyder S., Huerta-Cepas J., Simonovic M., Doncheva N.T., Morris J.H., Bork P., Jensen L.J., Mering C.V. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res*. 2019; 47(D1):D607-D613. DOI 10.1093/nar/gky1131.

Tanguy E., Kassas N., Vitale N. Protein-phospholipid interaction motifs: A focus on phosphatidic acid. *Biomolecules*. 2018;8(2):20. DOI 10.3390/biom8020020.

Tanguy E., Wolf A., Wang Q., Chasserot-Golaz S., Ory S., Gasman S., Vitale N. Phospholipase D1-generated phosphatidic acid modulates secretory granule trafficking from biogenesis to compensatory endocytosis in neuroendocrine cells. *Adv. Biol. Regul*. 2022;83:100844. DOI 10.1016/j.jbior.2021.100844.

Ta-Shma A., Zhang K., Salimova E., Zernecke A., Sieiro-Mosti D., Stegner D., Furtado M., Shaag A., Perles Z., Nieswandt B., Rein A.J., Rosenthal N., Neiman A.M., Elpeleg O. Congenital valvular defects associated with deleterious mutations in the *PLD1* gene. *J. Med. Genet*. 2017;54(4):278-286. DOI 10.1136/jmedgenet-2016-104259.

Teramoto R., Tada H., Kawashiri M.A., Nohara A., Nakahashi T., Konno T., Inazu A., Mabuchi H., Yamagishi M., Hayashi K. Molecular and functional characterization of familial chylomicronemia syndrome. *Atherosclerosis*. 2018;269:272-278. DOI 10.1016/j.atherosclerosis.2017.11.006.

Torres M.M.T., Mora-Hernández S., Vázquez Cárdenas N.A., González Jaimes A. Homozygous familial hypercholesterolemia: the c.1055G>A mutation in the *LDLR* gene and clinical heterogeneity. *J. Clin. Lipidol*. 2014;8(5):525-527. DOI 10.1016/j.jacl.2014.05.002.

Vaezi Z., Amini A. Familial Hypercholesterolemia. In: StatPearls. StatPearls Publishing: Treasure Island, FL, USA, 2022. Available online: https://www.ncbi.nlm.nih.gov/books/NBK556009/ (accessed on 24 July 2022).

Valdes V.J., Athie A., Salinas L.S., Navarro R.E., Vaca L. CUP-1 is a novel protein involved in dietary cholesterol uptake in *Caenorhabditis elegans*. *PLoS One*. 2012;7(3):e33962. DOI 10.1371/journal.pone.0033962.

Vasilyev V., Zakharova F., Bogoslovskaya T., Mandelshtam M. Familial hypercholesterolemia in Russia: three decades of genetic studies. *Front. Genet*. 2020;11:550591. DOI 10.3389/fgene.2020.550591.

Wiegman A., Gidding S.S., Watts G.F., Chapman M.J., Ginsberg H.N., Cuchel M., Ose L., … Santos R.D., Stalenhoef A.F., Steinhagen-Thiessen E., Stroes E.S., Taskinen M.R., Tybjærg-Hansen A., Wiklund O., European Atherosclerosis Society Consensus Panel. Familial hypercholesterolaemia in children and adolescents: gaining decades of life by optimizing detection and treatment. *Eur. Heart J*. 2015;36(36):2425-2437. DOI 10.1093/eurheartj/ehv157.

World Health Organization-Human genetics DoNDP. Familial hypercholesterolaemia: report of a second World Health Organization consultation, Geneva: WHO; 1999. URL: https://apps.who.int/iris/handle/10665/66346 (Application: 14.11.2022).

Wuni R., Kuhnle G.G.C., Wynn-Jones A.A., Vimaleswaran K.S. A nutrigenetic update on *CETP* gene-diet interactions on lipid-related outcomes. *Curr. Atheroscler. Rep*. 2022;24(2):119-132. DOI 10.1007/s11883-022-00987-y.

Zakharova F.M., Damgaard D., Mandelshtam M.Y., Golubkov V.I., Nissen P.H., Nilsen G.G., Stenderup A., Lipovetsky B.M., Konstantinov V.O., Denisenko A.D., Vasilyev V.B., Faergeman O. Familial hypercholesterolemia in St.-Petersburg: the known and novel mutations found in the low density lipoprotein receptor gene in Russia. *BMC Med. Genet*. 2005;6:6. DOI 10.1186/1471-2350-6-6.

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