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RESEARCH COMMUNICATION

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A novel variant in *LPL* gene is associated with familial combined hyperlipidemia

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Abstract

Familial combined hyperlipidemia (FCHL) is a common genetic disorder characterized by increased fasted serum cholesterol, triglycerides, and apolipoprotein B-100. Molecular genetic techniques such as next generation sequencing have been very successful methods for rare variants finding with a moderate-to large effect. In this study, we characterized a large pedigree from MASHAD study in northeast Iran with coinheritance of FCHL and early-onset coronary heart disease. In this family, we used whole-exome sequencing and Sanger sequencing to determine the disease-associated gene. We identified a novel variant in the *LPL* gene, leading to a substitution of an asparagine for aspartic acid at position 151. The D151N substitution cosegregated with these characters in all affected family members in the pedigree but it was absent in all unaffected members in this family. We speculated that the mutation D151N in *LPL* gene might be associated with FCHL and early-onset coronary heart disease in this family. However, the substantial mechanism requires further investigation.

KEYWORDS

cholesterol, familial combined hyperlipidemia, FCHL, *LPL* gene, triglycerides

1 | INTRODUCTION

Familial combined hyperlipidemia (FCHL) is a common genetic disorder associated with elevated fasted serum total cholesterol, triglyceride (TG), or both.[1,2] FCHL is one of the

Abbreviations: apoB, apolipoprotein B-100; CHDs, coronary artery diseases; FCHL, familial combined hyperlipidemia; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; NGS, next generation sequencing; VLDL, very low density lipoprotein; WES, whole-exome sequencing.

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most common metabolic diseases with prevalence rate from 0.5 to 2% worldwide known as a major risk factor for cardiovascular diseases.[2,3]

Main characteristics of FCHL are including increased plasma small very low density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL) levels, decreased high-density lipoprotein levels, and increased apolipoprotein B-100 (apoB), which is the major apolipoprotein for chylomicrons, VLDL, IDL, and LDL particles, and this could be due to either an increase in apoB production or a decrease in its catabolism.[4,5] This oligogenic lipid disorder occurs due to the interaction of some contributing variants and mutations associated with environmental triggers. Therefore, the genetic background of FCHL remains unknown.[6–8]

Modern, molecular genetic techniques have an important role in determining the genes involved in a huge number of diseases.[9–12] The emergence of a new generation of sequencing technique as named "next generation sequencing (NGS)" with high-speed, high-power, and low-cost sequencing has provided grounds for identifying genes and variables with high-to-moderate effect on disease.[11] These methods are particularly effective in families with several affected members with a particular disorder.[13,14] In this study, we used whole-exome sequencing (WES) and Sanger sequencing techniques to investigate a large pedigree with a reoccurring pattern of FCHL associated with early onset heart disease and high blood pressure.

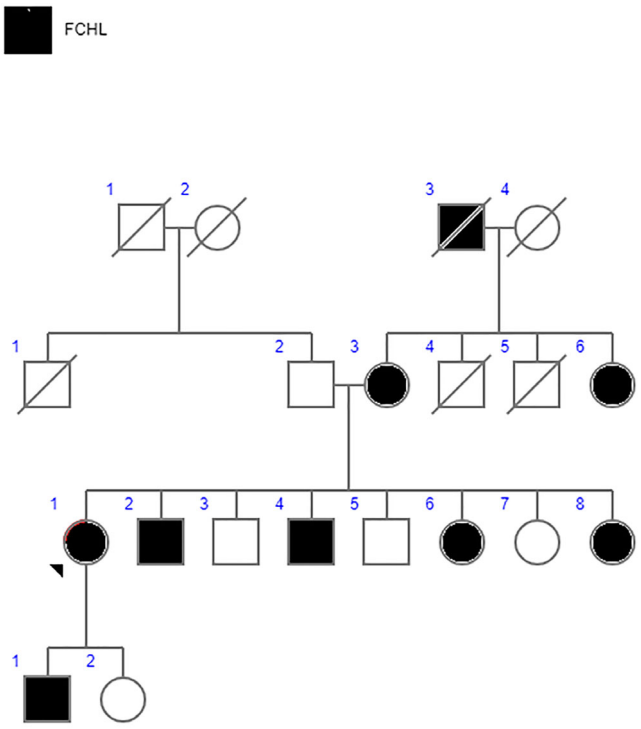

FIGURE 1 Pedigree of a family with FCHL associated with early-onset coronary artery disease. Circles indicate female family members, and squares male family members; slashes indicate that the family member is deceased. Family members with FCHL disease are indicated by solid symbols, and those without FCHL indicated by open symbols. FCHL, familial combined hyperlipidemia

2 | MATERIAL AND METHODS

2.1 | Study population

The protocol of this study was approved by the local ethics committee of Mashhad University of Medical Sciences. In addition, all study participants of this study signed consent form. We identified a family with a reoccurring familial pattern of FCHL from a cohort called MASHAD study[15] in northeast Iran which they had an unusual constellation of juvenile-onset FCHL and early-onset coronary heart disease. They had elevated fasting serum TG and cholesterol levels and high blood pressure. FCHL diagnosis was confirmed for them in according 2016 ESC/EAS Guidelines for dyslipidemias. In this family, there were several affected members with the FCHL phenotype and early-onset coronary artery disease (CHD) with a presumed autosomal dominant inheritance pattern (Figure 1). After evaluation these family members, we collected clinical and laboratory data (Table 1) for all members of this family who were more than 30 years old. In addition, we collected 5 ml blood contain EDTA anticoagulant and then, genomic DNA was extracted. For biochemical analysis, we collected 5 ml blood without anticoagulant and after 30 min, we separated serum with centrifuge at 5000 RPM for 10 min at room temperature.

2.2 | Whole-exome sequencing

The index patient who is identified by arrow in Figure 1 was screened with WES. This technique was used to sequence all exons of protein-coding regions and some important other genomic regions. WES carried out by using Illumina Sequencer with 100 million reads. In total, in this platform, >95% of the targeted regions examined with an acceptable sensitivity about 99%. WES has a capability for identifying microinsertion/deletions, point mutations, and duplication (<20 bp) in exons and some flanked sequences in targeted regions. After this, bioinformatics analysis carried out by using international databases and some bioinformatics softwares. In this step, we filtered raw output, to remove common variants that are present in databases and reference genomes. We also applied filters against published databases and those variants with deleterious effects on the proteins were selected. These variants usually change the conservation and tissue expression of proteins and they have novel effect on proteins. We used confirmation steps in some international databases such as OMIM, GeneCards, and MalaCards. Also, we used prediction softwares such as SIFT, Mutation Taster, FATHMM, and DANN to prediction effects of missense variant on the biological function of protein.

TABLE 1 The clinical characteristics of the affected and unaffected members

No ID	Age/age of onset CHD	FBG	Chol	LDL	TG	Smoking	HTN
II-3	46	82	305	186	201	No	Yes
II-6	38	79	290	187	184	No	Yes
III-1	35	83	301	192	189	No	Yes
III-2	41	99	295	194	202	No	Yes
III-3	Not affected	92	202	121	115	No	No
III-4	38	73	265	192	211	No	Yes
III-5	Not affected	110	175	120	101	No	No
III-6	38	91	275	200	189	No	Yes
III-7	Not affected	88	162	129	98	No	No
III-8	48	76	269	171	201	No	Yes
IV-1	35	85	312	271	198	No	Yes
IV-2	Not affected	85	167	122	118	No	No

Abbreviations: CHD, coronary heart disease; Chol, total cholesterol; FBG, fasting blood glucose; HTN, hypertension; LDL, low density lipoprotein; TG, triglyceride.

TABLE 2 Characteristics of primers for polymerase chain reaction (PCR) and Sanger sequencing

Primers (5′->3′)	Product size (bp)	Features associated with this product
Forward primer ACGGAAAAGTGAAACAAAAGA	461	Lipoprotein lipase precursor
Reverse primer GCCCTACAATGAGATAATCAAGTGC		

2.3 | Variant validation studies with Sanger sequencing

After WES annotation, we found a variant of undetermined significance in exon 4 *LPL* gene. *LPL* gene mutations have previously been found to be associated with FCHL in previous studies. After WES, we confirmed this variant in the index patient by PCR technology and Sanger sequencing technique (PCR primers and their characteristics are shown in Table 2). In addition, segregation studies carried out for other affected and nonaffected members of this pedigree. PCR was performed using mastermix contains a solution of DNA polymerase, deoxyribonucleotide triphosphates, reaction buffer and $MgCl_2$, deoxyribonucleotide triphosphates, Taq DNA polymerase and 10 pM of forward and reverse primers, 100 ng DNA, and reactions were done in a volume of 25 µl. PCR was done in an initial denaturation at 95°C for 5 min followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 59°C for 45 s and extension at 72°C for 1 min and a final extension at 72°C for 10 min. We checked PCR products with electrophoresis on agarose gel (Figure 2) and then, products were sent for sequencing by using the Sanger sequencing technique. We analyzed the gene sequences using Snap gene software.

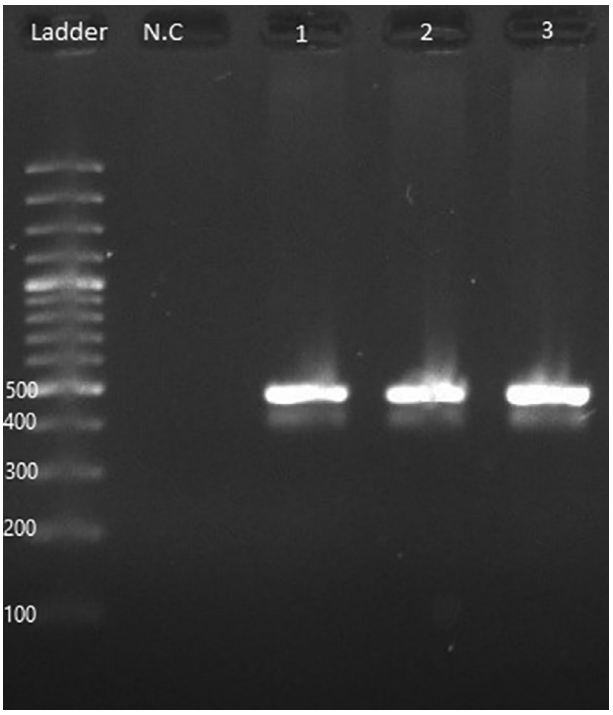

FIGURE 2 A PCR product with 461 by length. N.C, negative control; 1, 2, and 3 are the product PCR for Sanger sequencing

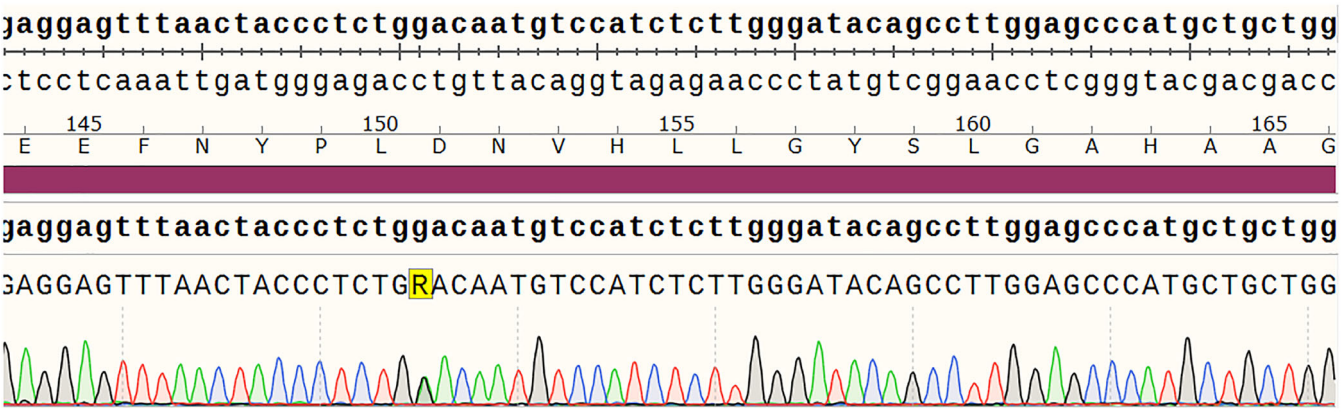

FIGURE 3 DNA sequence of a segment flanking D151 in *LPL* gene from an affected member. A single base substitution changes a guanine to adenine, leading to the substitution of acid aspartic (D) for glutamine (N) at codon 151. R denotes a heterozygote C→T nucleotide substitution

TABLE 3 Some features of D151N mutation *LPL* gene in prediction softwares

No.	Prediction software	Status
1	Mutation taster	Disease causing
2	DANN	Damaging
3	MetalR	Damaging
4	SIFT	Tolerated
5	FATHMM	Damaging
6	FATHMM-MKL	Damaging
7	PROVEAN	Neutral
8	DANN	Damaging

3 | RESULTS

The clinically characterized members of this family included seven affected and who had CHDs before Age 50. All affected family members had total cholesterol >240 mg/dl, LDL > 160 mg/dl, and TG > 133 mg/dl and according to the 2016 ESC/EAS guidelines associated with management of dyslipidemias, they had the standard criteria for FCHL.

After filtering of the WES data in the index, patient common single nucleotide polymorphisms (SNPs) that were found in databases as nonpathogenic and variants with frequency more than 1% were excluded. We identified a rare protein-altering variant in the index patient in *LPL* gene on chromosome 8 in all affected members of this pedigree. This mutation was absent in all unaffected members of this pedigree. This mutation is a substituted asparagine for acid aspartic at position 151 of *LPL* gene (p. D151N) (Figure 3). In addition, this variant is conserved among orthologues and paralogues in some species include Mmusculus, Xtropicalis, and humans. This variant is a disease-causing variant in prediction softwares such as Mutation taster, FATHMM, and DANN (Table 3).

4 | DISCUSSION

FCHL is a common form of hyperlipidemia worldwide and is related to increased risk of early-onset CHD associated with increased VLDL and LDL levels.[16,17] Affected individuals have an elevation of both cholesterol and TGs in the blood.[18] Our study showed a variant in *LPL* gene which may be related to FCHL and can be determinant in the development of the disease in patients who had early-onset CHD in a pedigree with several affected members. The *LPL* gene, which encodes the enzyme lipoprotein lipase, plays an important role in breaking down lipids in the form of TGs which are carried by lipoproteins molecules from some various organs to the blood.

Until now, scientists determined about 40 mutations in the *LPL* gene related to lipoprotein lipase deficiency and familial chylomicronemia disease.[1,19]

A previous study has shown that individuals heterozygote for lipoprotein lipase deficiency can develop FCHL and a defect in the *LPL* gene can occur in up to a fifth of FCHL families.[20] Yang et al. showed that one in 20 FCHL patients was compound heterozygous for mutations in the promoter region of the LPL.[19] Also previous studies showed that decreasing activity of *LPL* gene was present in approximately one-third patients with FCHL.[17] Therefore, it seemed that LPL levels reducing, due to mutation in LPL alleles, together with several factor(s) is a cause in patients with FCHL.[19]

So far, more than 30 different FCH-related genes have been identified, each with a different level of scientific evidence and this list is expanding.[7]

Past studies have shown that some variations in the *LPL* gene affect blood lipid levels.[21–23] Hoffer et al. showed a common mutation in the *LPL* gene (Asn291Ser) which was related to elevated lipid levels and this variation probably is one of the main genetic factors predisposing to FCHL in the families who were studied.[24] Also in another study by

Gagne et al., they reported four variants in a French Canadian FCHL. They used single-strand conformation polymorphism technique and they showed that two of these variants were inactive catalytically.[25] There are also some variations in *LPL* gene that we can divide them to rare and common variants which are related to FCHL. Rare variants are including p. Asp277Asn in exon 6, p. Thr379Ala in exon 7, and p. Val397Met known as rs298.[26,27] Common variants include p. Asp36Asn (rs1801177)[28] which increases the LDL by five times in vitro, p.Asn318Ser(rs268),[29] p. Ala427Thr(rs5934),[26] and p.Ser474*(rs328).[30]Variants in *LPL* gene result in a change in the production of lipoprotein lipase enzymes that may have altered abilities to break down TGs.[23] When this enzyme is overactive, there is a low lipid level and in other cases that the enzyme is impaired, leadings to increased plasma lipids.[31] Individuals with hyperlipidemia have a greater than normal risk for developing atherosclerosis.[32] Fatty plaque accumulates over time, eventually blocking the arteries and increase the risk having a stroke and heart attack.[32] Although the pivotal role of LPL in developing lipid disorders has been suggested, the exact mutation analysis of this gene in patients with dislipidemic disorders such as FCHL is not fully investigated. On the other hand, the due the multifactorial basis of dislipidemia, a large number of genetic and environmental factors appear to determine the risk of developing atherosclerosis in sporadic (isolated) dislipedemic patients.[33] Minicocci et al. showed that loss-of-function in *LPL* gene, APOA5 gene, and GCKR gene, through interaction with several variants in other genes and can lead to elevated TG levels and increase LDL-C concentrations in FCHL patients.[26]

Based on previous studies, another gene related to FCHL is *LDLR* gene. In a review study done by Minicocci et al. in 2015, they listed a table of rare and common variants in *LDLR* genes related to FCHL. They determined eight different sequence variations with heterozygous state in patients with FCHL. In addition, they identified a variant in the *LDLR* gene in heterozygous state known as p. Ala391Thr (rs11669576) although they were not doing a functional study for this variant.[26] We did not find any deleterious variant in the *LDLR* gene in present study and this why it was not reported in the text section.

Another study was done by Hayden et al. and showed an association between FCH and an XmnI RFLP in *APOA1* gene which was in linkage with AI-CIII-AIV cluster on 11q23-q24.[34]

In a study by Coon et al., they found two SNPs in *USF1* gene which were associated with FCHL LDL cholesterol, and TGs in pedigrees from 87 Utah including early death due to early-onset hypertension, early strokes, and coronary heart disease.[35] In another study done by Di Taranto et al., they showed that polymorphisms in *APOA5* gene such as S19W was associated with FCHL and total cholesterol, TGs, and BMI in 165 FCH patient.[36] Also Huertas-Vazquez et al. in a study showed an association between FCH characteristics such as TG, apoB and total cholesterol (TC) levels, and a nonsynonymous variant in *PCDH15* gene from Caucasian families.[37]

Our study showed a novel missense mutation in *LPL* gene exon 4 (p. D151N) and we conclude that this mutation in *LPL* gene may be related to a clinical syndrome that is characterized by elevation cholesterol, TG, and CHD. However, the substantial mechanisms should be further investigated. The emergence of the NGS techniques suggests molecular sequencing assessments in large cohorts of FCHL to determine the full genetic background of FCHL.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

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