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LDLR c.89_92dup: a novel frameshift variation in familial hypercholesterolemia

Jialing Deng^{1,2†}, Ju Zhang^{1,2†}, Shirui Meng³, Nan Ding^{1,2}, Yu Hao^{1,2}, Hui Zeng^{1,2*} and Jie Lin^{4*}

Abstract

Background Familial hypercholesterolemia (FH) is a common inherited metabolic disease that causes premature atherosclerosis, cardiovascular disease, and even death at a young age. Approximately 95% of FH-causing genetic variants that have been identified are in the *LDLR* gene. However, only 10% of the FH population worldwide has been diagnosed and adequately treated, due to the existence of numerous unidentified variants, uncertainties in the pathogenicity scoring of many variants, and a substantial number of individuals lacking access to genetic testing.

Objective The aim of this study was to identify a novel variant in the *LDLR* gene that causes FH in a Chinese family, thereby expanding the spectrum of FH-causing variants.

Methods Patients were recruited from Beijing Anzhen Hospital, Capital Medical University. FH diagnosis was made according to the Dutch Lipid Clinical Network (DLCN) criteria. Whole-exome sequencing (WES) was conducted to identify the FH-causing variant in the proband, and amplicon sequencing was used to verify the variant in his family members.

Results A three-generation Chinese family was recruited, and two FH patients were clinically diagnosed, both without known FH-causing variants. These two FH patients and another possible patient carried a novel variant, NC_00019.9(NM_000527.5):c.89_92dup (NP_000518.1:p.Phe32Argfs*21), in the ligand-binding domain of the low-density lipoprotein (LDL) receptor that led to a frameshift. The FH adults in the family showed severe clinical symptoms and statin therapy resistance.

Conclusion This study identified a novel pathogenic *LDLR* variant, c.89_92dup, associated with severe FH clinical manifestations and statin therapy resistance.

Keywords Familial hypercholesterolemia, Low-density lipoprotein receptor, c.89_92dup variant, Ligand-binding domain, Whole-exome sequencing

[†]Jialing Deng and Ju Zhang contributed equally to this work.

*Correspondence:

Hui Zeng

zenghui@ccmu.edu.cn

Jie Lin

linjie1998@126.com

¹Biomedical Innovation Center, Beijing Shijitan Hospital, Capital Medical University, Beijing 100038, China

²Beijing Key Laboratory for Therapeutic Cancer Vaccines, Beijing 100038, China

³Queen Mary School, Jiangxi Medical College, Nanchang University, Nanchang 330006, China

⁴Department of Atherosclerosis, Beijing Anzhen Hospital, Beijing Institute of Heart, Lung and Blood Vessel Diseases, Capital Medical University, Beijing 100029, China



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Background

Familial hypercholesterolemia (FH) is an autosomal dominant metabolic disorder that is characterized by high serum levels of low-density lipoprotein (LDL) cholesterol [1]. The prevalence of FH is approximately 1 in 250~500 worldwide [2, 3], which is higher than that of most other inherited diseases. In patients carrying heterozygous variants, serum LDL cholesterol levels are typically above 5.0 mmol/L, which is 2 times greater than those in unaffected individuals within the same family [4]. In patients carrying homozygous variants, serum LDL cholesterol levels frequently exceed 13.0 mmol/L [5]. Long-term hypercholesterolemia can manifest as tendon xanthomas and arcus cornealis [1]. When left untreated, FH patients often suffer from premature atherosclerosis, cardiovascular disease (CVD), and even death at a very young age [6, 7]. In China, individuals with FH have a 15-fold increased risk of developing CVD compared to those without FH [8].

Known FH-causing variants are found in several genes that are associated with the removal of LDL particles from the circulation by hepatocytes, such as *LDL receptor (LDLR, >95%)*, *apolipoprotein B (APOB, 2–11%)*, and *proprotein convertase subtilisin/kexin type 9 (PCSK9, <1%)* [1, 9]. The *LDLR* gene is located at 19p13.2 and comprises 18 exons spanning 45 kilobases (kb). It encodes an 860 amino acid protein that consists of five functional domains, including a ligand-binding domain, an epidermal growth factor (EGF) precursor homology domain, an O-linked polysaccharide domain, a transmembrane domain, and an intracellular domain [10]. According to the statistical data from the ClinVar database [11] (<http://www.ncbi.nlm.nih.gov/clinvar/>), more than 40% of FH-causing variants are located in the EGF precursor homology domain, which impairs lipoprotein release and receptor recycling [12]. Another 40% of variants are located in the ligand-binding domain and therefore influence the binding of lipoproteins to LDL and VLDL (very low-density lipoprotein) [13]. No more than 20% of variants are scattered across the other three domains and introns [14].

According to the Familial Hypercholesterolemia Foundation and the World Heart Federation, only 10% of the FH population has been diagnosed and adequately treated [15]. Diagnoses are missed partially due to the existence of numerous unidentified variants, uncertainties in the pathogenicity scoring of many variants, and a substantial number of individuals lacking access to genetic testing. In this study, a three-generation Chinese FH family was diagnosed using the Dutch Lipid Clinical Network (DLCN) criteria [16]. A novel frame-shift variant, c.89_92dup, was identified in the ligand-binding domain of the LDLR protein via whole-exome sequencing (WES). This variant broadens the spectrum

of FH-causing variants, facilitating the diagnosis of individuals with FH.

Materials and methods

Study subjects

The subjects were recruited from an FH cohort at Beijing Anzhen Hospital, Capital Medical University. The proband was hospitalized and clinically diagnosed with FH according to the DLCN diagnostic criteria. Then, cascade screening was conducted for his family members. Six participants were included in the study, and all the subjects underwent a detailed physical examination. This study has been granted approval by the Ethics Committee of Beijing Anzhen Hospital (2022038X). All participants willingly took part in the research and provided their signatures on informed consent forms.

Isolation of peripheral blood mononuclear cells (PBMCs)

Peripheral blood was collected from the proband and his family members into Vacutainer tubes containing EDTA-2 K and processed for PBMC isolation in a short time. Following plasma extraction by centrifugation (2000 rpm, 10 min), the precipitate was resuspended in an equal volume of phosphate-buffered saline (PBS). The sample was then layered onto Ficoll-Paque (GE Healthcare, Marlborough, MA, USA) and subsequently processed in accordance with the manufacturer's instructions. DNA was extracted from approximately 1×10^6 PBMCs for genetic analysis.

Biochemical analysis

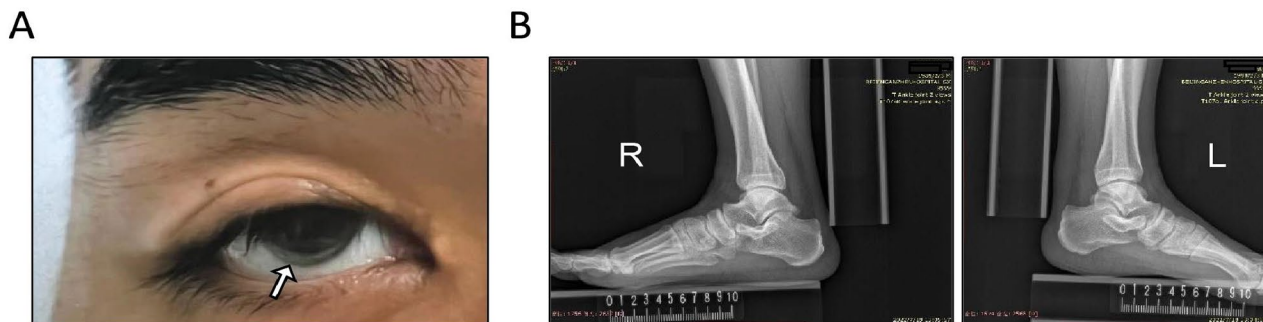
A Roche COBAS 701 analyzer was used to assess the serum total cholesterol (TC), total triglyceride (TG), high-density lipoprotein (HDL) cholesterol, non-HDL cholesterol, low-density lipoprotein (LDL) cholesterol, small dense LDL cholesterol, lipoprotein (a), apolipoprotein A1, and apolipoprotein B levels. The LDL cholesterol level was measured using a direct assay (LDL-Cholesterol Gen.3 (LDLC3), Roche Diagnostics).

WES library preparation and sequencing

A Qiagen DNeasy kit (Qiagen, Hilden, Germany) was used to extract genomic DNA from the peripheral blood cells of the proband. After sonication, the DNA fragments were subjected to end repair and were supplemented with adapters at both ends. Following library amplification, the fragments were captured and enriched using an exon array. After washing to remove the non-enriched fragments, the remaining DNA was amplified and subjected to whole-exome sequencing using the Illumina HiSeq 3000 platform in paired-end sequencing mode (analysis performed by the Guangzhou KingMed Center for Clinical Laboratory).

Table 1 DLCN scores of participants in the studied family

	Score	I.1	I.2	II.1(proband)	III.1	III.2
Family history						
First-degree relative with tendon xanthoma and/or arcus cornealis or children < 18 years of age with LDL cholesterol > 95th percentile by age and sex for country	2	2	2	2	2	2
First-degree relative with known premature (< 55 years of age in men, < 60 years of age in women) coronary heart disease or known low-density lipoprotein (LDL) cholesterol > 95th percentile by age and sex for country	1					
Clinical history						
Patient has premature coronary heart disease	2	2		2		
Patient has premature cerebral or peripheral arterial disease	1					
Physical examination						
Presence of tendon xanthoma	6			6		
Presence of arcus cornealis in a patient < 45 years of age	4					
LDL cholesterol						
≥ 8.5 mmol/L	8					
6.5–8.4 mmol/L	5	5		5		
5.0–6.4 mmol/L	3					
4.0–4.9 mmol/L	1				1	
Total score		9	2	15	3	2
Classification		Definitive	Unlikely	Definitive	Possible	Unlikely

**Fig. 1** Clinical manifestations of the proband. Physical examination revealed corneal arcus (A) and tendon xanthomas (B) in the proband

The raw reads were mapped to the reference human genome (hg19) using Bowtie2 [17]. After filtering low-quality nucleotides (quality < 30) and reads with lengths less than 18 bp by Trimmomatic [18], the qualified reads were further processed with SAMtools to call SNPs using mpileup [19]. SNPs detected on all known genes were annotated with ANNOVAR software [20].

Amplicon sequencing

Primers I and II were designed according to the *LDLR* sequence in the GenBank nucleotide sequence database (<https://www.ncbi.nlm.nih.gov/nucleotide/>) and had the following sequences: Primer I, 5'- TTTATTAATGTGC ATGGAAGTTCT-3'; and Primer II, 5'- AGACCAGAA ATTCAAGACCAGC-3'. With polymerase chain reaction (PCR) technology, amplicons containing the variant were amplified from equal amounts of DNA samples. Sanger sequencing was employed to validate the identified variant in other members of the family. Amino acid

conservation near the variant position was examined using MEGA 11.0 software.

Open reading frame prediction of the *LDLR* variant

The NCBI ORF Finder (<https://www.ncbi.nlm.nih.gov/orffinder>) was used to identify changes in the open reading frame (ORF) for the NM_000527.5 sequence with the c.89_92dup variant.

Results

Clinical characteristics of the FH family

The proband was a 32-year-old man who was hospitalized for “unstable angina” and clinically diagnosed with FH according to the DLCN criteria (Table 1). The patient showed severe clinical symptoms of FH, including arcus cornealis and tendon xanthoma (Fig. 1). Coronary angiography revealed triple-vessel disease involving the right coronary artery (RCA), left circumflex artery (LCX), and left anterior descending artery (LAD), with

complete occlusion in the distal segment of the circumflex artery. The patient was insensitive to statin therapy. Before treatment, his LDL cholesterol level reached 8.12 mmol/L. After two years of treatment with 20 mg atorvastatin plus 10 mg ezetimibe, his LDL cholesterol level remained high at 6.92 mmol/L. Furthermore, he suffered an acute myocardial infarction while on the lipid-lowering regimen. Fortunately, the patient responded well to a PCSK9 inhibitor. After one month of treatment with evinacumab (140 mg every 2 weeks), his LDL cholesterol level decreased to 0.85 mmol/L.

Upon investigation of the proband's family history, it was discovered that his father (I.1) also met the clinical criteria for FH (Table 1), with an untreated LDL cholesterol level of 6.75 mmol/L. In his forties and again in his fifties, the father underwent arterial stent implantation for acute coronary syndrome (ACS). The elder daughter (III.1) of the proband was seven years old and exhibited significantly higher levels of LDL cholesterol (4.29 mmol/L). Neither the proband's mother (I.2) nor the younger daughter (III.2) exhibited FH-related clinical symptoms. Table 2 shows the family's lipid profile and clinical manifestations.

Variant identification in the FH family

In the proband (II.1), a novel variant, c.89_92dup, was identified in exon 2 of the *LDLR* gene by WES (Fig. 2A). No variants were identified in other FH-causing genes

Table 2 Clinical characteristics of the proband and his family members

Family Members	Father I.1	Mother I.2	Proband II.1	Elder daughter III.1	Younger daughter III.2
Sex	Male	Female	Male	Female	Female
Age (years)	57	57	32	7	5
Triglycerides (mmol/L)	1.14	0.75	1.06	0.86	0.68
Total cholesterol (mmol/L)	9.19	5.55	10.18	6.03	4.34
HDL cholesterol (mmol/L)	1.58	1.22	1.06	1.11	1.69
LDL cholesterol (mmol/L)	6.75	3.66	8.12	4.29	2.08
Non-HDL cholesterol (mmol/L)	7.61	4.33	9.12	4.92	2.65
Small dense LDL cholesterol (mmol/L)	1.37	0.53	1.52	0.57	-
Lipoprotein (a) (nmol/L)	53.3	91.7	38.5	51.6	8.6
Apolipoprotein A1 (g/L)	1.55	1.34	1.13	1.2	1.58
Apolipoprotein B (g/L)	1.91	1.13	2.04	1.23	0.72
Coronary artery disease	Yes	No	Yes	No	No

LDL: low-density lipoprotein; HDL: high-density lipoprotein

or other exons of the *LDLR* gene. This duplication variant was located in a phylogenetically conserved region of the ligand-binding domain (Fig. 2B) and produced a frameshift in the coding sequence. The frameshift ORF included an arginine amino acid variant at the 32nd position, which replaced the wild-type phenylalanine, and translation terminated at the 52nd amino acid (Fig. 2C). By predicting potential ORFs longer than 50 codons in the *LDLR* variant, four other ORFs were discovered to include novel translation start codons (TSCs, Fig. 2C). The longest predicted protein was truncated at the 263rd amino acid and lacked the first five repeats and a portion of the sixth repeat of the ligand-binding domain of LDLR.

Variant segregation analysis

Amplicon sequencing confirmed that the c.89_92dup variant was carried by the proband's father (I.1) and elder daughter (III.1), while the proband's mother (I.2) and younger daughter (III.2) had the wild-type *LDLR* gene. Figure 3 displays the pedigree of this FH family, showing that dyslipidaemia was correlated with the segregation arrangement of the frameshift *LDLR* variant (Table 1). Therefore, the c.89_92dup variant was determined to be the pathogenic variant for this FH family.

Discussion

This study reported a family in which the proband and his father were clinically diagnosed with FH according to the DLCN criteria, and the proband's elder daughter exhibited significantly higher levels of LDL cholesterol than her contemporaries. WES and amplicon sequencing confirmed that these three patients were found to carry the same variant, c.89_92dup, which was consistent with autosomal dominant inheritance. This duplication variant was situated within the ligand-binding domain of LDLR, which consists of seven repeats of 40 residues each and contains the binding sites for apoproteins B-100 (apoB-100) and E (apoE) [21, 22]. These apoproteins are essential components of LDL and VLDL particles. Since this frameshift variant is absent from control populations (Genome Aggregation Database, 1000 Genomes Project, and Human Gene Mutation Database) [23–25] and segregated with a phenotype in 3 informative meioses in the proband's family, the c.89_92dup variant was classified as pathogenic (PVS1, PM2, PP1 and PP4) according to the ACMG guidelines [26, 27].

Five ORFs in the *LDLR* gene with the c.89_92dup variant were found to be longer than 50 amino acids. Among them, ORF1 had the same TSC as the wild-type *LDLR* transcript. This ORF potentially encoded a truncated protein consisting of only 32 amino acids at the N-terminus of LDLR, along with 20 novel amino acids from the frameshift. Since dozens of RNA splice sites are located downstream of the premature termination codons (PTCs)

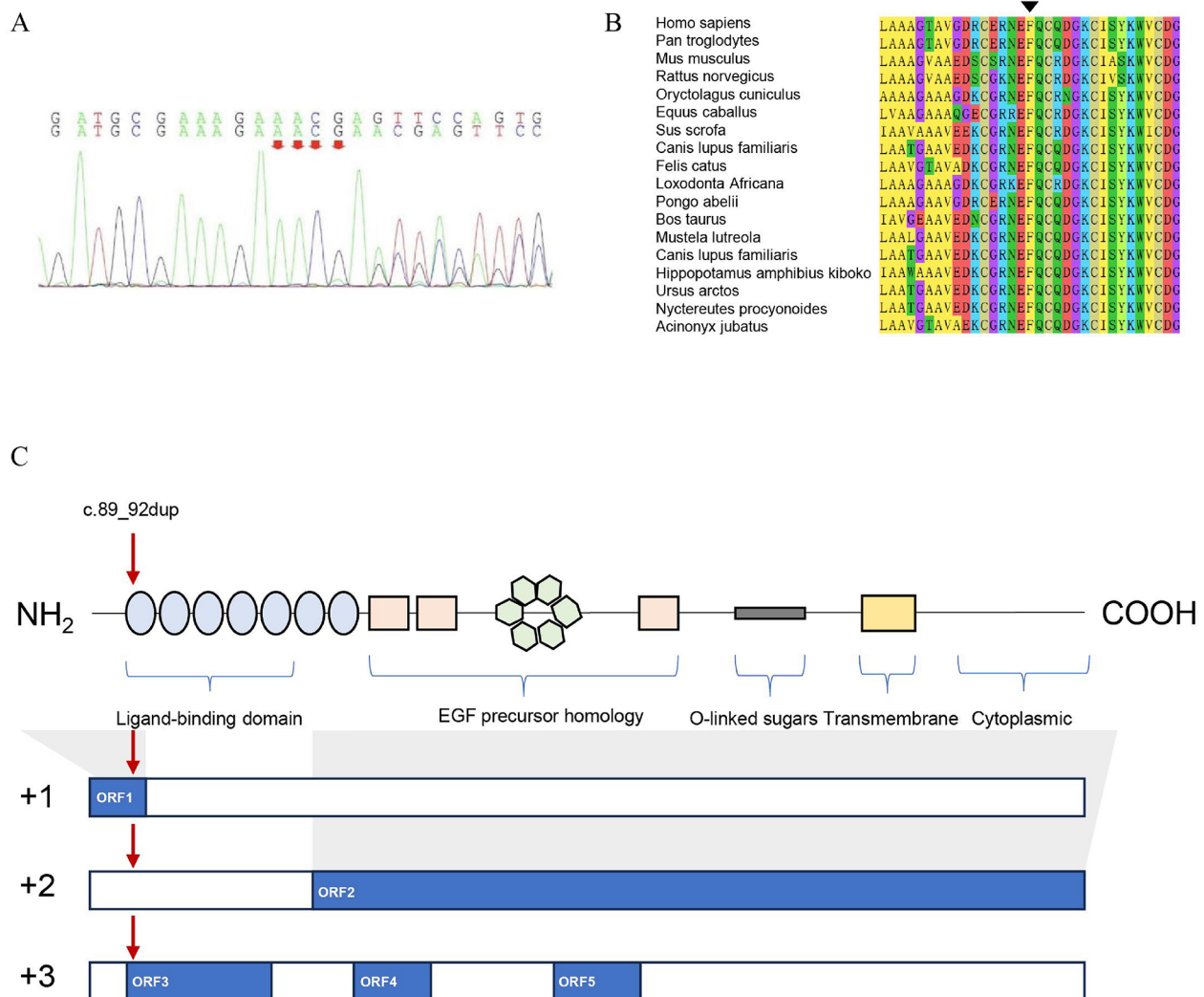


Fig. 2 Confirmation of the c.89_92dup frameshift variant of *LDLR*. **(A)** Sequence chromatogram showing the c.89_92dup variant of *LDLR* in the proband. The variant was numbered according to GenBank NM_000527.5. **(B)** Alignment of mammalian *LDLR* proteins showing that the regions around the variant are highly conserved. The position of the variant is marked by a black triangle. **(C)** Model showing five domains in the structure of the human *LDLR* protein. The blue rectangles display the results of ORF prediction. The red arrow indicates the location of the variant carried by the proband

in ORF1, the unremoved exon-exon junction complexes (EJCs) binding to mRNA should trigger nonsense-mediated mRNA decay (NMD) to degrade the transcript. ORF2 was predicted to produce an N-terminal truncated protein, adopting an ATG codon at positions 794–796 of the wild-type *LDLR* coding sequence (CDS) as the TSC. This truncated protein lacked the first five repeats and a portion of the sixth repeat of the ligand-binding domain of *LDLR*. The fifth repeat is required to bind β -VLDL, and the second, third, and sixth repeats are required for maximal binding of LDL [13]; therefore, the encoded protein produced by ORF2 lacks most of its ligand-binding functions. Additionally, the amino acid sequences encoded by the other three potential ORFs (ORF3–5) do not resemble those of the wild-type *LDLR* protein. In

summary, although the impact of the c.89_92dup variant on *LDLR* protein sequence and abundance still needs to be verified, this variant may severely impair *LDLR* function in patients.

Consistent with the ORF analyses, patients with the c.89_92dup variant had more severe clinical symptoms. In the reported FH family, the proband and his father had LDL cholesterol levels of 8.12 mmol/L and 6.75 mmol/L, respectively. These findings were significantly greater than the median level of 5.43 mmol/L (IQR: 4.32–6.72 mmol/L) observed in adults with heterozygous FH who did not use lipid-lowering medications [4]. In addition, the proband suffered an acute myocardial infarction at the early age of 32, while his father had a myocardial infarction in his forties and a recurrent episode of ACS at

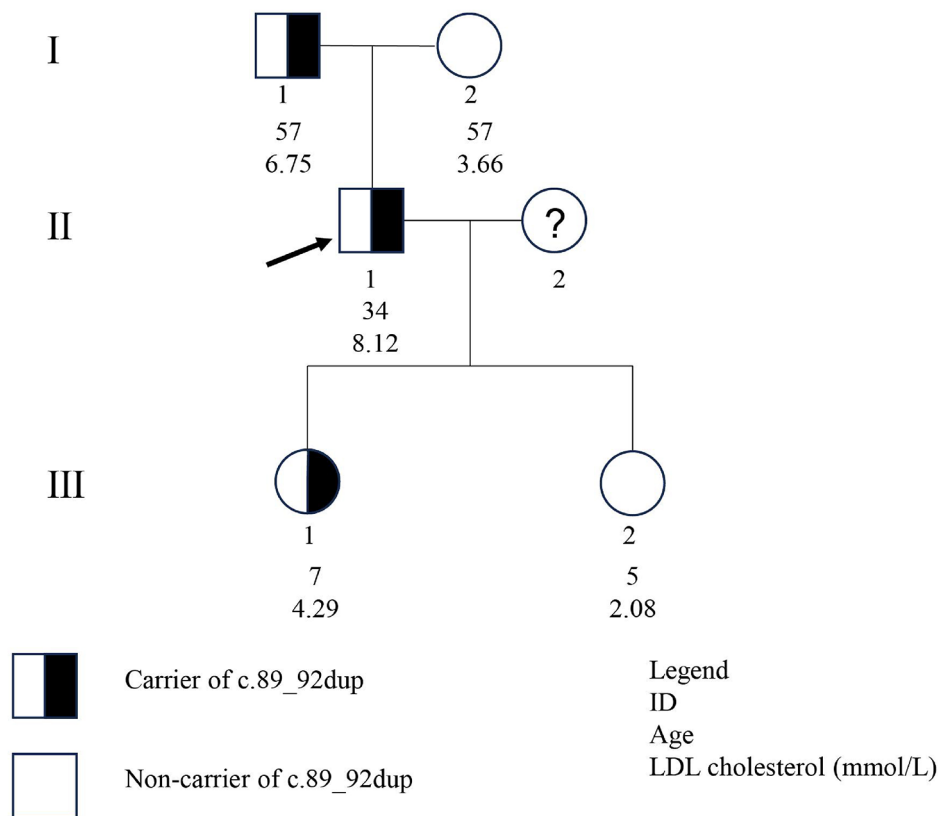


Fig. 3 Pedigree of the family showing the segregation of the *LDLR* variant: ex2: c.89_92dup: p.(Phe32fs). The arrow indicates the proband; circle and square symbols represent women and men, respectively

the age of 55. Both patients met the diagnostic criteria for severe FH [28].

More importantly, patients with the c.89_92dup variant seemed to be insensitive to statin therapy. Here, the proband's LDL cholesterol level decreased by only 15% after two years of treatment with 20 mg atorvastatin plus 10 mg ezetimibe. This observation further showed that the duplication variant significantly impaired LDLR function, resulting in an ineffective reduction in circulating cholesterol levels despite inhibiting cholesterol synthesis and absorption. However, the proband responded well to a PCSK9 inhibitor. When taken with statins and ezetimibe, coadministration of a PCSK9 inhibitor reduced the patient's LDL cholesterol level by up to 90%. Because a heterozygous FH individual has the homologous wild-type *LDLR* gene, a PCSK9 inhibitor increases LDLR abundance on the surface of hepatocytes, thus enhancing its ability to clear LDL cholesterol [29, 30]. Therefore, PCSK9 inhibitors are recommended for lipid-lowering therapy in patients carrying the *LDLR* c.89_92dup variant at an early stage.

Strengths and limitations of the study

Using WES and variant segregation analysis, a frameshift variant, c.89_92dup, was identified as a novel pathogenic variant for severe FH with a poor response to statin therapy. This finding contributes to the clinical diagnosis and treatment of FH patients. Further molecular testing of the residual activity of this *LDLR* variant could provide valuable insights into its biological functions.

Conclusions

The current study identified a novel pathogenic *LDLR* variant, c.89_92dup, associated with severe FH clinical manifestations and statin therapy resistance. This study expands the spectrum of FH-causing variants and provides assistance for disease screening and individualized treatment. For optimal management, FH patients with the c.89_92dup variant should receive PCSK9 inhibitors in a timely manner.

Abbreviations

FH	familial hypercholesterolemia
DLCN	Dutch Lipid Clinical Network
LDLR	low-density lipoprotein receptor
APOB	apolipoprotein B
PCSK9	proprotein convertase subtilisin/kexin type 9
WES	whole-exome sequencing

LDL	low-density lipoprotein
TG	triglycerides
TC	total cholesterol
HDL	high-density lipoprotein
CVD	cardiovascular disease
EGF	epidermal growth factor
PBMCs	peripheral blood mononuclear cells
ORF	open reading frame
RCA	right coronary artery
LCX	left circumflex artery
LAD	left anterior descending artery
ACS	acute coronary syndrome
TSC	translation start codon
APOE	apolipoprotein E
PTC	premature termination codon
EJC	exon junction complex
NMD	nonsense-mediated mRNA decay
CDS	coding sequence

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12944-024-02173-2>.

Supplementary Material 1

Supplementary Material 2

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Author contributions

J.D. and J.Z. drafted the manuscript. J.D. and S.M. collected the clinical data. J.Z., N.D. and Y.H. performed the genetic analyses and the bioinformatic analysis. J.L. interpreted the clinical data. H.Z. and J.L. supervised and revised of the manuscript. All authors gave final approval of the submitted version.

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Data availability

The variation data that support the findings of this study have been deposited in the Genome Variation Map in National Genomics Data Center, China National Center for Bioinformation / Beijing Institute of Genomics, Chinese Academy of Sciences, under accession number PRJCA023739.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Beijing Anzhen Hospital, Capital Medical University (2022038X). All subjects voluntarily participated in the study and signed informed consent forms.

Competing interests

The authors declare that they have no competing interests.

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