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Targeted Genetic Analysis in a Chinese Cohort of 208 Patients Related to Familial Hypercholesterolemia

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Aim: Familial hypercholesterolemia (FH) is the most commonly encountered genetic condition that predisposes individuals to severe autosomal dominant lipid metabolism dysfunction. Although more than 75% of the European population has been scrutinized for FH-causing mutations, the genetic diagnosis proportion among Chinese people remains very low (less than 0.5%). The aim of this study was to identify genetic mutations and help make a precise diagnosis in Chinese FH patients.

Methods: We designed a gene panel containing 20 genes responsible for FH and tested 208 unrelated Chinese possible/probable or definite FH probands. In addition, we called *LDLR* copy number variation (CNVs) with the panel data by panelcn.MOPS, and multiple ligation-dependent probe amplification (MLPA) was used to search for CNVs in *LDLR*, *APOB*, and *PCSK9*.

Results: A total of 79 probands (38.0%) tested positive for a (likely) pathogenic mutation, most of which were *LDLR* mutations, and three *LDLR* CNVs called from the panel data were all successfully confirmed by MLPA analysis. In total, 48 different mutations were identified, including 45 *LDLR* mutations, 1 *APOB* mutation, 1 *ABCG5* mutation, and 1 *APOE* mutation. Among them, the five most frequent mutations (*LDLR* c.1879G>A, c.1747C>T, c.313+1G>A, c.400T>C, and *APOB* c.10579C>T) were detected. Moreover, we also found that patients with *LDLR* variants of CNVs and splicing and nonsense had increased low-density lipoprotein cholesterol levels when compared with those who carried missense variants.

Conclusions: The spectrum of FH-causing mutations in the Chinese population is refined and expanded. Analyses of FH causal genes have been a great help in clinical diagnosis and have deep implications in disease treatment. These data can serve as a considerable dataset for next-generation sequencing analysis of the Chinese population with FH and contribute to the genetic diagnosis and counseling of FH patients.

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Key words: Familial hypercholesterolemia, Chinese, Genetic testing, CNVs

Introduction

Familial hypercholesterolemia (FH, OMIM #143890) is an autosomal dominant disorder characterized by an increased level of circulating low-density lipoprotein cholesterol (LDL-C) that leads to lipid accumulation in skin, tendons, and arteries; premature atherosclerosis; and increased risk of cardiovascular disease[1, 2]. The prevalence of FH is estimated as high as 1 in 200–500[3], with even higher frequencies in populations with founder effects[4]. FH is also the first genetic disorder shown to cause myocardial infarction[5], leading to premature heart disease and death in affected individuals. If untreated, men have a 50% chance of coronary heart disease before the age of 50 years, and women have a 30% risk by the age of 60 years[6, 7].

Most FH cases are caused by mutations in three

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main genes, namely, low-density lipoprotein receptor (*LDLR* 90%), apolipoprotein B (*APOB* 5%–10%), and proprotein convertase subtilisin/kexin type 9 (*PCSK9* 1%–2%)[8]. Previous studies revealed that the FH mutation detection rate for definite FH patients varies between 63% and 87%[9, 10], suggesting that there are other genetic causes, located outside of the currently screened regions, yet to be identified. The importance of identifying an FH-causing variant, which has clinical utility in providing an unequivocal diagnosis[11], has been emphasized by the National Institute of Health and Care Excellence, which in 2008 recommended cascade testing using deoxyribonucleic acid (DNA) information for finding the affected relatives of a patient[12].

Considering the fact that traditional approaches such as direct sequencing have difficulty identifying other genetic causes in addition to the three main genes, a high-throughput and effective method to detect genetic defects is needed. Thus, we designed customized 20 genes involved in FH genetic disease in next-generation sequencing (NGS) platforms in this study. To identify genetic mutations, make a precise diagnosis, and establish an FH genetic database for the Chinese population, we recruited 208 probands with definite or possible/probable FH in Fuwai Hospital and performed gene panel testing in those patients who related to FH phenotypes. Furthermore, the performance of CNVs from our panel data was also evaluated. Herein, we report the molecular findings from 208 patients, which, to our knowledge, is the first group of FH panel detection ever reported in China.

Aim

In this study, we designed a gene panel containing 20 genes responsible for FH and tested 208 unrelated probands. Our aim is to identify genetic mutations and provide assistance for the genetic diagnosis and counseling of FH patients.

Methods

Patients and Consent

The study was approved by the ethics committee of Fuwai Hospital and adhered to the Declaration of Helsinki. All experimental protocols were approved by the ethics committee of Fuwai Hospital and were carried out in accordance with the approved guidelines. All of the patients enrolled in this study were from Fuwai Hospital. Each individual who underwent the genetic test was adequately informed regarding the benefits and risks of the test and signed the consent form.

Between February 2015 and April 2018, we consecutively recruited 208 unrelated Chinese possible/probable or definite FH patients. Clinical data and baseline characteristics were collected prospectively by a trained nurse from medical records and by direct interview of patients. The diagnosis of FH was established using the Dutch Lipid Clinic Network (DLCN) criteria[3]. The following numerical score definition of FH was employed: family history of a first-degree relative with known premature coronary artery disease (pCAD <55 years for men; <60 years for women) or vascular disease and/or a first-degree relative with known hypercholesterolemia (1 point) or tendon xanthoma (2 points) or offspring(s) with known hypercholesterolemia (2 point); personal history of pCAD (ages as above, 2 points) or premature cerebral or peripheral vascular disease (ages as above, 1 point) or tendon xanthoma (6 points); and LDL-C level higher than 325 mg/dl (8 points), 251–325 mg/dl (5 points), 191–250 mg/dl (3 points), or 155–190 mg/dl (1 point). Notably, individuals on lipid-lowering medications with their pretreatment LDL-C unavailable had their untreated LDL-C levels conservatively adjusted by a relative correction factor that depended on the dose and potency of the statin according to previous studies[13, 14]. A diagnosis of FH was considered definite if the total score was greater than 8, probable if the score was 6–8, possible if the score was 3–5, and unlikely if the score was below 3 points. We did not employ the criteria relating to corneal arcus and molecular genetic testing to define the FH phenotype in the algorithm. Finally, the patients with a DLCN score of >3.0 were included for genetic testing.

Gene panel Testing

A custom-designed gene panel containing 20 genes **(Table 1)** known to be associated with the FH syndrome was ordered from Life Tech, USA, with coverage of 99.7% of the target regions. Genomic DNA was extracted from ethylenediaminetetraacetic acid-anticoagulated whole blood and checked to assure the quality and quantity before processing. Library preparation was performed according to the manufacturer's instructions (Ion AmpliSeq™ Library Kit 2.0, Life Technologies, Inc.). Pooled libraries (up to 20 samples per chip) were sequenced on the Ion 318™ Chip on Life PGM™ instrument. Suspected pathogenic variants were confirmed using Sanger sequencing.

Bioinformatics Analysis

Only high-quality reads were retrieved by filtering out low-quality reads and adaptor sequences using Trimmomatic software. The clean-read sequences were aligned to the human reference genome (hg19) by

Table 1. FH panel genes

Gene	Locus	Protein	Disease	Exons	Amplicons	Coverage
LDLR	19p13.2	low density lipoprotein receptor	Familial hypercholesterolemia	18	29	0.997
LPL	8p21.3	lipoprotein lipase	Hyperchylomicronemia	10	19	1
APOB	2p24.1	apolipoprotein B	Familial hypercholesterolemia	31	63	1
LDLRAP1	1p36.11	low density lipoprotein receptor adaptor protein 1	Hypercholesterolemia	14	19	1
EPHX2	8p21.2-p21.1	epoxide hydrolase 2	Hypercholesterolemia	19	21	1
PPP1R17	7p14.3	protein phosphatase 1, regulatory subunit 17	Hypercholesterolemia	5	9	1
GHR	5p13.1-p12	growth hormone receptor	Hypercholesterolemia	18	34	1
ABCG5	2p21	ATP binding cassette subfamily G member 5	Sitosterolemia	15	17	1
ABCG8	2p21	ATP binding cassette subfamily G member 8	Sitosterolemia	15	18	1
APOE	19q13.32	apolipoprotein E	Hyperlipoproteinemia	6	9	1
APOC2	19q13.32	apolipoprotein C2	Hyperlipoproteinemia	4	6	1
ITIH4	3p21.1	inter-alpha-trypsin inhibitor heavy chain 4	Hypercholesterolemia	24	30	1
PCSK9	1p32.3	proprotein convertase subtilisin/kexin type 9	Familial hypercholesterolemias.	14	24	1
CETP	16q13	cholesteryl ester transfer protein	Hyperalphalipoproteinemia	17	19	1
MTTP	4q23	microsomal triglyceride transfer protein	Abetalipoproteinaemia	19	24	1
ABCA1	9q31.1	ATP binding cassette subfamily A member 1	HDL deficiency	53	65	1
APOA5	11q23.3	apolipoprotein A5	Hyperchylomicronemia	4	11	1
APOC3	11q23.3	apolipoprotein C3	Hyperalphalipoproteinemia	4	5	1
SCARB1	12q24.31	scavenger receptor class B, member 1	High density lipoprotein cholesterol level quantitative trait locus 6;	13	21	1
APOA2	1q23.3	apolipoprotein A2	Hypercholesterolemia	4	4	1

Burrows-Wheeler Aligner, and PCR duplicates were marked by Picard software. SNPs and insertions/deletions were identified using the GATK HaplotypeCaller program (http://www.broadinstitute.org/gsa/wiki/index.php/Home_Page) and further annotated with comprehensive ANNOVAR software for their frequencies in the Genome Aggregation Database (gnomAD) for the pathogenicity and splicing-altering prediction of single nucleotide variants in the dbNSFP database, which included results from SIFT, Ployphen-2, MutationTaster, the dbscSNV database. In addition, the clinical significance of the sequences was annotated using ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/), OMIM (http://omim.org/), UniProt (http://www.uniprot.org/), and HGMD (http://www.hgmd.org). Variants with cutoff values greater than 0.6 in the dbscSNV database were defined as splice altering. Other synonymous variants that did not fulfill the abovementioned conditions were removed. Considering that most genes related to FH were inherited in an autosomal dominant manner, variants with a minor allele frequency (MAF) of >0.5% were

Table 2. Clinical characteristics of the study population according to the presence of putative (likely) pathogenic mutation

	Total (n = 208)	Mutation (+) (n = 79)	Mutation (–) (n = 129)
Sex (% male)	52.9	54.2	52
Age, y	48.3 ± 11.2	45.1 ± 11.0	50.6 ± 10.6
pCAD, % (n)	61.1 (127)	92.4 (73)	41.9 (54)
Lipid-lowering therapy, % (n)	53.4 (111)	89.9 (71)	31 (40)
Xanthomas, % (n)	4.3 (9)	11.4 (9)	0 (0)
TG, mg/dl	76.4 ± 45.8	84.0 ± 76.4	87.9 ± 68.8
TC, mg/dl	427.8 ± 61.1	446.9 ± 110.8	389.6 ± 80.2
HDL-C, mg/dl	45.8 ± 11.5	38.2 ± 7.6	47.8 ± 13.0
LDL-C, mg/dl	324.7 ± 53.5	340.0 ± 87.9	234.2 ± 63.4
Definite FH, % (n)	56.7 (118)	72.2 (57)	47.3 (61)
Possible/probable FH, % (n)	43.3 (90)	27.8 (22)	52.7 (68)

excluded from further analysis and regarded as polymorphism variants.

Variant Classification

Variants were analyzed for pathogenicity according to the recommendations of the American College of Medical Genetics. Specifically, the analysis was based on the following criteria: (i) whether they were previously reported by a functional study or family segregation study; (ii) the nature of the variant (e.g., nonsense, frameshift indel, or splicing mutations (intron ±1 or ± 2)); (iii) variant frequency in population databases; (iv) conservation of the altered residue; (v) in silico prediction (SIFT, PholyPhen2, or MutationTaster); (vi) de novo mutation; and (vii) family segregation studies. On the basis of this information, a variant was classified into one of the five following categories: benign, likely benign, unknown significance, likely pathogenic, or pathogenic[15].

Copy Number Variation Calling

All CNVs were called by panelcn.MOPS[16], which was designed to detect targeted NGS panel data. Sequencing quality was checked and duplications were removed by Picard software before CNV calling. Samples with a high correlation of read counts were selected automatically as controls from all the samples by panelcn.MOPS. Other parameters were kept default.

Multiplex Ligation-Dependent Probe Amplification (MLPA)

MLPA assays were performed to detect *LDLR*, *APOB*, and *PCSK9* large deletions or duplications using the commercially available SALSA MLPA Kits P062 (MRC-Holland, Amsterdam, The Netherlands), which contained probes for all exons of *LDLR*, *APOB*, and *PCSK9*. According to the manufacturer's instructions, a total of 100–200 ng of genomic DNA from each patient was used for hybridization, and amplification products from each MLPA assay were separated by capillary electrophoresis on an ABI 3500XL Dx Genetic Analyzer (Life Technologies, USA). The results were analyzed using Coffalyser software.

Results

Clinical Characteristics

The basic clinical statistical data are shown in **Table 2**. The mean age of the probands was 48.3 ± 11.2 years, and 110 probands (52.9%) were females. pCAD was noted in 127 patients (61.1%), and tendon xanthoma in 9 patients (4.3%). The mean ± standard deviation of total cholesterol (TC) and LDL-C was 427.8 ± 61.1 and 324.7 ± 53.5 mg/dl, respectively. According to the DLCN criteria, 56.7% of individuals had been diagnosed with definite FH, whereas 43.3% were diagnosed with possible/probable FH **(Table 2)**. The mutation-positive group (likely pathogenic or pathogenic mutation) showed obviously higher TC (446.9 ± 110.8 vs. 389.6 ± 80.2) and LDL-C (340.0 ± 87.9 vs. 234.2 ± 63.4) levels than did the mutation-negative group. The number of (likely) pathogenic mutations detected also increased in xanthoma and pCAD patients when compared with that in the mutation-negative group.

Molecular Findings of FH

Sequencing of the 20 FH genes **(Table 1)** in the 208 samples yielded a mean depth of ~400X and coverage of 98.5%. On the basis of sequencing results, 79 (38.4%) of the patients were positive for a (likely) pathogenic mutation, 29 (13.9%) had a VUS, and 100 (48.1%) were tested negative using the 20-gene FH panel. Most of the (likely) pathogenic mutations were located in the *LDLR* gene. In addition, to raise

the diagnostic rate, we also conducted CNV calling with panel data using panelcn.MOPS, which is a newly developed pipeline to detect CNVs in targeted NGS panel data for clinical diagnostics[16]. In this process, a few filtering conditions were needed to reduce the false-positive rate of the preliminary results. We considered the sample as negative if the *LDLR* gene had several discontinuous deletions. Finally, we found that probands FH008 and FH-9 harbored large *LDLR* deletions of exons 2–8 and exons 7–12, respectively, and FH-10, a large *LDLR* duplication of exons 3–6. To confirm the results, we performed MLPA not only in these 3 probands but also in those 18 probands who had severe phenotypes and undetected by gene panel sequencing. As a result, the three *LDLR* CNVs were confirmed **(Fig. 1 and Table 3)**, suggesting that the method implemented by panelcn.MOPS was highly effective for *LDLR* CNV detection.

In total, 48 different (likely) pathogenic mutations were identified, including 45 *LDLR* mutations (6 novel mutations), 1 *APOB* mutation, 1 *ABCG5* mutation, and 1 *APOE* mutation, with no detection of *PCSK9* (likely) pathogenic mutations **(Table 4)**. The pathogenicity of mutations in other genes, including *PCSK9*, was difficult to define because of the lack of functional studies or strong family segregation evidence. In this study, we also found the five most frequent (likely) pathogenic mutations (*LDLR* c.1879G>A, c.1747C>T, c.313+1G>A, c.400T>C, and *APOB* c.10579C>T), with a total frequency of 12.0% in all examined patients, or 31.6% in the (likely) pathogenic mutation group.

Genotype–Phenotype Correlation between FH Patients with Different LDLR Mutations

We investigated the genotype–phenotype correlation between *LDLR* mutation type and LDL-C level in these FH patients. Of the 208 probands, 47 had missense mutations, 20 had nonsense plus in-frame deletions, 9 had splicing mutations, and 3 had abnormal MLPA patterns of *LDLR*. We attempted to study the correlation between *LDLR* mutation type and LDL-C level in those patients, and the results are listed in **Table 5**. These results suggested that patients with *LDLR* variants of CNVs and splicing and nonsense had increased LDL cholesterol (LDL-C) levels when compared with those who carried missense variants (351.4±225.4 vs. 217.7±156.6, 259.8±129.9 vs. 217.7±156.6, and 236.8±57.3 vs. 217.7±156.6, respectively). Furthermore, the LDL-C level of patients with *LDLR* variants of CNVs was the highest among these different carriers of *LDLR* mutation type.

Discussion

Heterozygous FH was traditionally thought to have a prevalence of 1 in 500; however, data now suggest a higher frequency[17]. In fact, FH is vastly underdiagnosed, and there are several reasons for this: First, LDL-C levels and other clinic presentations of FH are variable[18]; second, a small family size may obscure the inherited nature of FH; and third, with FH being only one of multiple genetic and exogenous conditions affecting CAD risk, it might be overlooked in the large number of CAD/MI patients. Prevalence estimates from studies conducted in Australia[19] (n=18,322), China[20] (n=9,324), and the USA[21] (National Health and Nutrition Examination Survey; n=36,949) were 1 in 229 to 1 in 350, 1 in 322, and 1 in 250, respectively. In view of the large proportion of potential FH patients in China and the low rate of genetic confirmation with suspected FH patients, an efficient and economical method for detecting gene defects is needed. Thus, we designed a gene panel containing 20 genes responsible for FH that have been utilized in some laboratories[6, 22] and then tested 208 unrelated probands in this study. Our aim is to assess the frequency of mutations caused by FH in the Chinese population and to provide assistance for the genetic diagnosis and counseling of FH patients. To the best of our knowledge, this is the first FH panel test ever reported in China.

The results showed that there were 48 different mutations detected in the *LDLR*, *APOB*, *ABCG5*, and *APOE* genes. The five most frequent (likely) pathogenic mutations detected in our study were *LDLR* c.1879G>A, c.1747C>T, c.313+1G>A, c.400T>C, and *APOB* c.10579C>T, with a total frequency of 12.0% in all examined patients, or 31.6% in the (likely) pathogenic mutation group. Given the relatively high frequency of these five variants detected in the Chinese population and to reduce the cost of conventional genotyping, the microarray, including the five different spots of *LDLR* and *APOB*, may be an effective and cost-efficient way to widespread mass screening in the general population. For patients without mutations detected by this rapid genetic screening, all cases with clinical definite/probable FH or some selected cases with possible FH phenotypes can undergo further comprehensive sequencing and MLPA analysis. Among these five mutations, the c.1879G>A; p.Ala627Thr mutation in the *LDLR* gene is the most frequent mutation in this study (3.8%), which is in agreement with previous studies[2]. Sun *et al.* reported that this mutation affected the binding of LDL to its receptor and exhibited 50% mature protein in transfected COS cells[23]. The

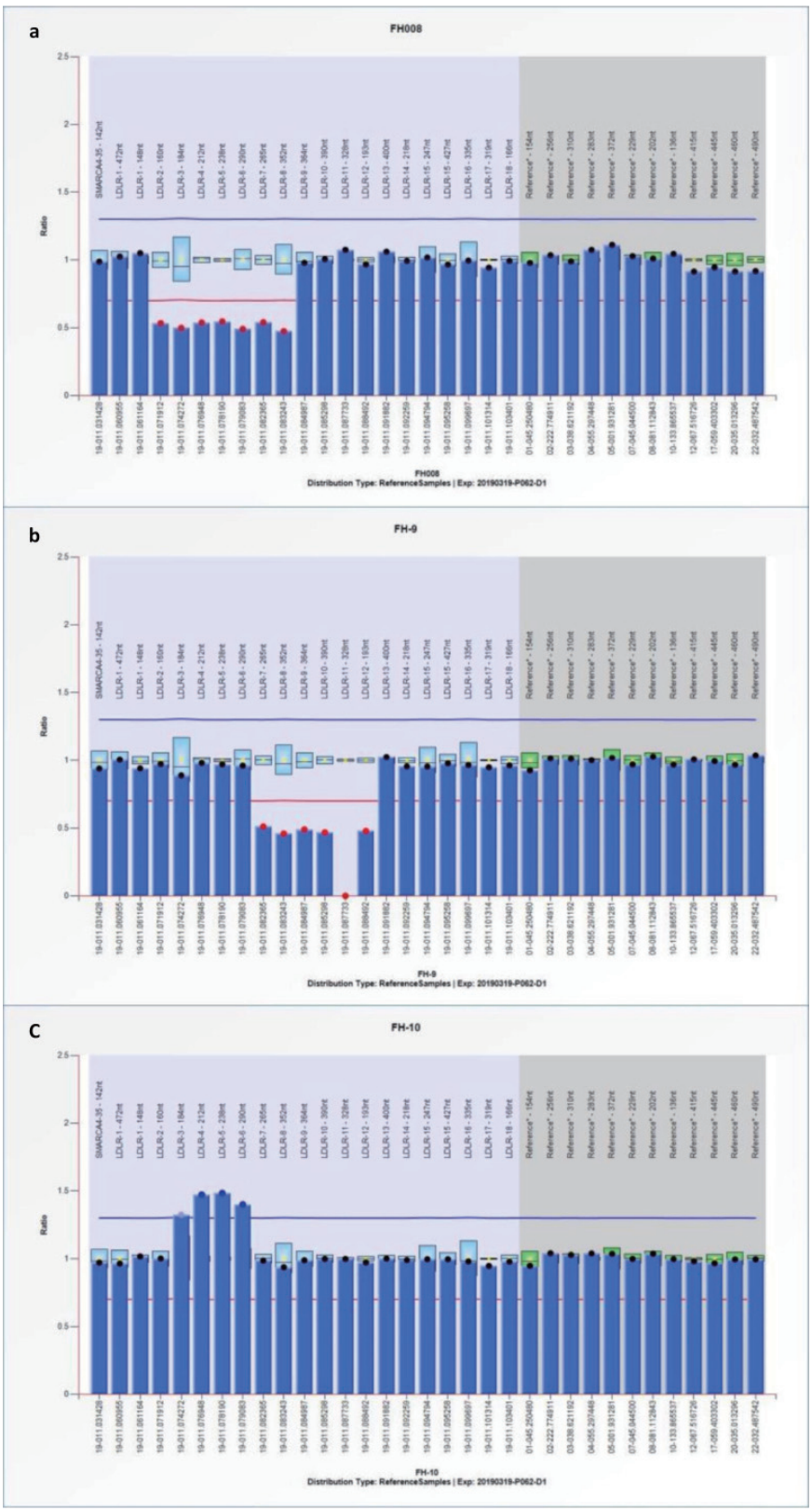

Fig. 1. Results of semiquantitative MLPA for three patients

(a) Reduced relative peak areas of *LDLR* exons 2–8 for patient FH008. (b) Reduced relative peak areas of *LDLR* exons 7–12 for patient FH-9. (c) Increased relative peak areas of *LDLR* exons 3–6 for patient FH-10.

Table 3. CNVs in *LDLR* by panelcn.MOPS and MLPA

Patient No.	Phenotype	Gene	Transcript	panelcn.MOPS	MLPA	Accordance
FH008	Probable FH	*LDLR*	NM_000527	Ex2-8 del, het	Ex2–8 del, het	Full
FH-9	Definite FH	*LDLR*	NM_000527	Ex7-12 del, het	Ex7–12 del, het	Full
FH-10	Definite FH	*LDLR*	NM_000527	Ex3-6 dup, het	Ex3–6 dup, het	Full

Ex, exon; het, heterozygous; dup, duplication; del, deletion

NM_000384: c.10579C>T; R3527W variant in the *APOB* gene has been reported previously in association with hypercholesterolemia using alternate nomenclature, R3500W[24]. The R3527W variant is observed in 11 of 8,642 (0.1%) alleles from individuals of East Asian background in large population cohorts[25]. Functional studies demonstrated that the R3527W variant reduced *APOB* capacity for binding, uptake, and degradation of LDL[26]. Furthermore, a different missense variant affecting the same residue (R3527Q) has been reported in the Human Gene Mutation Database in association with an *APOB*-related disorder[27]. The NM_000527: c.313+1G>A variant in the *LDLR* gene destroys the canonical splice donor site in intron 3 and is predicted to result in abnormal splicing of the *LDLR* message. Functional studies have shown that the c.313+1G>A variant disrupts mRNA splicing and produces a protein with abnormal function[28]. The NM_000527: c.400T>C change replaces cysteine with arginine at codon 134 of the LDLR protein (p.Cys134Arg). It affects a cysteine residue located within an LDLRA domain of the LDLR protein. Cysteine residues in these domains are involved in the formation of disulfide bridges, which are critical for protein structure and stability[29]. The c.1747C>T mutation replaces histidine with tyrosine at codon 583 of the LDLR protein (p.His583Tyr). Experimental studies have shown that this missense change impairs lipoprotein uptake by reducing the number of surface receptors[23]. In addition to the five most frequent mutations, we also detected six novel (likely) pathogenic mutations in the *LDLR* gene, including four frameshift variants, one splicing variant, and one nonsense variant. Interestingly, the *ABCG5* c.1336C>T (p.Arg446Term) and *APOE* c.461G>A (p.Arg154His) mutations were detected in an 8-year-old girl and a 47-year-old man, respectively, whose LDL-C levels were 373.4 and 359.1 mg/dl, respectively, which were relatively high when compared with those of *LDLR* mutation carriers. These two patients had no xanthomas or family history of cardiovascular disease. Loss of function mutations of *ABCG5* results in sitosterolemia, an autosomal recessive disorder in which there is increased fractional absorption and decreased biliary secretion of neutral sterols and contributed to the development of the FH phenotype, leading to the misdiagnosis of FH in some sitosterolemia patients[30, 31]. Tada *et al.* had shown that there were substantial proportion of the patients with hypercholesterolemia caused by *ABCG5* genetic mutation(s), and they suggested that rare mutations in *ABCG5* may, at least in some patients, mimic FH or exacerbate the FH phenotype[32]. Furthermore, previous study revealed that patients with *APOE* mutations also had the same phenotype as that of FH patients[33]. Therefore, the FH panel testing in such patients would help to identify variants that are associated with increased risk for future atherosclerotic cardiovascular disease events and provide valuable prognostic information that may be used to initiate appropriate preventive therapies.

As for the result of MLPA, three *LDLR* CNVs were indicated from the panel data and finally validated by MLPA, which has a 100% concordance rate with MLPA results. The ability to detect full-spectrum mutations in *LDLR* is critical for obtaining a molecular diagnosis of FH, especially since up to 10% or more of these mutations are large-scale CNVs rather than small-scale DNA sequence mutations[34]. The current procedure for diagnostic laboratories often includes targeted NGS followed by MLPA. Our findings suggest that the information about potential CNVs also resides within NGS data and that MLPA may be potentially dispensable for the *LDLR* gene. The indication of CNVs from panel data will greatly save the costs of testing specified samples.

A genotype–phenotype correlation between LDLR mutation type and LDL-C level in these FH patients was investigated. Interestingly, we found that patients with LDLR variants of CNVs and splicing and nonsense had increased LDL-C levels when compared with those who carried missense variants in this study **(Table 5)**. In addition, we also found that despite their similar FH-causing mutations, patient lipid levels were significantly different. In this regard, we believe that it may be caused by interacting genetic effects, such as large-effect variants[35], polygenic effects[36], gene–environment interactions (including the effects of diet and lifestyle)[37], or non-Mendelian mechanisms[38]. Moreover, the prevalence of tendon

Table 4. Pathogenic and likely pathogenic mutations detected in our cohort

Gene	Transcript	Exon/ Intron	Nucleotide Change	Protein Change	Pathogenicity	PMID/Novel	MAF	Proband No.
LDLR	NM_000527	Exon 1	c.12G>A	p.Trp4Term	Pathogenic	7903864	2.982×10^{-5}	1
LDLR	NM_000527	Exon 1	c.17G>A	p.Trp6Term	Pathogenic	Novel	NA	1
LDLR	NM_000527	Exon 2	c.81C>A	p.Cys27Term	Pathogenic	15556094	NA	1
LDLR	NM_000527	Exon 2	c.97C>T	p.Gln33Term	Pathogenic	1301940	1.791×10^{-5}	1
LDLR	NM_000527	Exon 2	c.138C>A	p.Cys46Term	Pathogenic	16806138	NA	1
LDLR	NM_000527	Exon 3	c.224G>A	p.Cys75Tyr	Likely pathogenic	9676383	NA	1
LDLR	NM_000527	Exon 3	c.268G>A	p.Asp90Asn	Likely pathogenic	9259195	7×10^{-4}	1
LDLR	NM_000527	Exon 3	c.285C>A	p.Cys95Term	Pathogenic	9852677	NA	1
LDLR	NM_000527	Exon 3	c.301G>A	p.Glu101Lys	Pathogenic	1301940	3.249×10^{-5}	1
LDLR	NM_000527	Intron 3	c.313+1G>A		Pathogenic	7718019	6.268×10^{-5}	4
LDLR	NM_000527	Exon 4	c.327C>A	p.Cys109Term	Pathogenic	NA	NA	1
LDLR	NM_000527	Exon 4	c.400T>C	p.Cys134Arg	Pathogenic	10735632	8.962×10^{-6}	4
LDLR	NM_000527	Exon 4	c.418G>T	p.Glu140Term	Pathogenic	1301956	3.249×10^{-5}	1
LDLR	NM_000527	Exon 4	c.510delC	p.Asp170fs	Likely pathogenic	Novel	NA	1
LDLR	NM_000527	Exon 4	c.622G>A	p.Glu208Lys	Likely pathogenic	1301956	NA	1
LDLR	NM_000527	Exon 4	c.682G>T	p.Glu228Term	Pathogenic	1301956	6.629×10^{-5}	2
LDLR	NM_000527	Exon 5	c.718G>A	p.Glu240Lys	Likely pathogenic	1301956	5.798×10^{-5}	2
LDLR	NM_000527	Exon 5	c.769C>T	p.Arg257Trp	Likely pathogenic	11462246	9×10^{-4}	3
LDLR	NM_000527	Intron 5	c.817+1G>C		Pathogenic	Novel	1.792×10^{-5}	2
LDLR	NM_000527	Intron 5	c.817+1G>A		Pathogenic	NA	NA	1
LDLR	NM_000527	Exon 7	c.974G>A	p.Cys325Tyr	Likely pathogenic	19318025	NA	1
LDLR	NM_000527	Exon 8	c.1135T>C	p.Cys379Arg	Likely pathogenic	1301956	NA	1
LDLR	NM_000527	Exon 9	c.1206delC	p.Phe402fs	Likely pathogenic	Novel	NA	1
LDLR	NM_000527	Exon 9	c.1222G>A	p.Glu408Lys	Likely pathogenic	1301956	10^{-4}	1
LDLR	NM_000527	Exon 9	c.1285G>A	p.Val429Met	Likely pathogenic	2569482	3.249×10^{-5}	1
LDLR	NM_000527	Exon 10	c.1448G>A	p.Trp483Term	Likely pathogenic	7903864	NA	2
LDLR	NM_000527	Exon 10	c.1474G>A	p.Asp492Asn	Likely pathogenic	9763532	10^{-4}	1
LDLR	NM_000527	Exon 10	c.1538delG	p.Arg513fs	Likely pathogenic	Novel	NA	2
LDLR	NM_000527	Exon 10	c.1567G>A	p.Val523Met	Likely pathogenic	2088165	3.249×10^{-5}	1
LDLR	NM_000527	Exon 11	c.1599G>A	p.Trp533Term	Pathogenic	10447263	NA	1
LDLR	NM_000527	Exon 11	c.1618G>A	p.Ala540Thr	Likely pathogenic	9544745	10^{-4}	2
LDLR	NM_000527	Exon 11	c.1633G>C	p.Gly545Arg	Likely pathogenic	NA	NA	1
LDLR	NM_000527	Intron 12	c.1706-1G>A		Pathogenic	16159606	NA	1
LDLR	NM_000527	Exon 12	c.1747C>T	p.His583Tyr	Likely pathogenic	7903864	1.3×10^{-3}	4
LDLR	NM_000527	Exon 12	c.1765G>A	p.Asp589Asn	Likely pathogenic	7903864	1.3×10^{-3}	2
LDLR	NM_000527	Exon 13	c.1864G>T	p.Asp622Tyr	Likely pathogenic	21377952	NA	3
LDLR	NM_000527	Exon 13	c.1879G>A	p.Ala627Thr	Likely pathogenic	7903864	5.798×10^{-5}	7
LDLR	NM_000527	Exon 13	c.1948delG	p.Glu650fs	Likely pathogenic	Novel	NA	1
LDLR	NM_000527	Exon 14	c.2054C>T	p.Pro685Leu	Pathogenic	2726768	6.536×10^{-5}	1
LDLR	NM_000527	Exon 16	c.2389G>A	p.Val797Met	Likely pathogenic	7649549	3.249×10^{-5}	1
LDLR	NM_000527	Intron 17	c.2390-2A>G		Pathogenic	8141835	NA	1
LDLR	NM_000527	Exon 17	c.2439G>A	p.Trp813Term	Pathogenic	3924410	NA	1
APOB	NM_000384	Exon 26	c.10579C>T	p.Arg3527Trp	Pathogenic	7627691	1.2×10^{-3}	6
ABCG5	NM_022436	Exon 10	c.1336C>T	p.Arg446Term	Likely pathogenic	17228349	9×10^{-4}	1
APOE	NM_000041	Exon 4	c.461G>A	p.Arg154His	Likely pathogenic	7706948	NA	1

NA, not available

Table 5. LDLR mutation type and mean average LDL-C in patients according to molecular diagnosis

Mutation Type	Number	Age (years)	Percent Female	Untreated LDL-C (mg/dl)
Splicing	9	39.8 ± 8.6	22.2	259.8 ± 129.9
Missense	47	55.3 ± 7.1	70.2	217.7 ± 156.6
Nonsense plus in-frame deletions	20	51.7 ± 11.2	20	236.8 ± 57.3
Abnormal MLPA pattern	3	45.3 ± 22.3	33.3	351.4 ± 225.4

xanthoma was only 4.3% in our study and was much lower than that reported in previous studies[39, 40]. Nevertheless, there was also a national FH screening program demonstrating that only 8% of affected relatives had xanthomas and only 5% had xanthelasma at the time of genetic testing[41]. They concluded that xanthoma, even though being a specific diagnostic criterion for FH, may not be a sensitive criterion. To our knowledge, whether a low prevalence of xanthoma is an ethnic characteristic of Chinese patients is not certain and will require further clarification.

The study has limitations that need to be acknowledged. First, this study did not check all individuals using MLPA. We conducted CNV calling with panel data to detect CNVs in targeted NGS panel data of all patients and found three *LDLR* CNVs. Then, MLPA was performed not only in these 3 probands but also in those 18 probands who had severe phenotypes and undetected by gene panel sequencing. As a result, the three *LDLR* CNVs were confirmed by MLPA. Second, a large number of patients in our cohort have been taking lipid-lowering drugs, and their untreated LDL-C levels needed to be adjusted by a relative correction factor that depended on the dose and potency of the lipid-lowering medications, which leads to their relatively high LDL-C levels. Third, the patients with a DLCN score of >3.0, which is the definition of possible FH, were included for genetic testing in this study. This soft inclusion criterion may be the cause of the low frequency of both mutation-positive FH and xanthomas.

Conclusion

Our data not only further expanded the *LDLR* mutation spectrum and provided evidence for the genotype–phenotype correlation given that FH patients with LDLR variants of CNVs and splicing and nonsense had increased LDL-C levels when compared with those who carried missense variants but also confirmed that the panelcn.MOPS pipeline is effective for detecting *LDLR* CNVs using panel sequencing data. Finally, our study provides and expands the scope of a valuable DNA database of FH gene mutations, which has great potential implications for the genetic diagnosis and counseling of FH patients.

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Conflicts of Interest

The authors have no conflicts of interest to declare.

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