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ORIGINAL ARTICLE

Expanding the genetic spectrum for Chinese familial hypercholesterolemia population with six genetic mutations identified using a next-generation sequencing-based laboratory-developed screening test

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Abstract

Background: This study was to reveal the prevalence of definite familial hypercholesterolemia (FH) in the hospital-visiting population, determine the pathogenic mutation detection rate in clinically diagnosed definite FH patients, and expand the FH mutation spectrum in China.

Methods: Blood lipid profiles of 41,803 patients visiting the hospital were investigated and 4967 patients with clinical diagnoses of other metabolic diseases were excluded. One hundred and seventy-three (0.41%) received a definite diagnosis of FH according to the Dutch Lipid Clinical Network Criteria-Chinese Revised Version (DLCN-CRV), and 18 patients subsequently agreed to undergo genetic testing. A next-generation sequencing (NGS)-based laboratory-developed test covering the exonic regions of 24 lipid metabolism-related genes was conducted alongside in silico analyses to identify possible FH mutations in 16 definite FH patients, according to the American College of Medical Genetics and Genomics (ACMG) criteria. Sanger sequencing was used to confirm mutations, and SWISS-MODEL was used to simulate the molecular structures of the confirmed protein-carrying mutations.

Results: The FH prevalence was 0.41% for the 41,803 individuals (DLCN-CRV grade >8) and 25% of definite FH patients carried six FH pathogenic mutations (≥ACMG Class 4). All genetic variants were confirmed by Sanger sequencing. Five pathogenic variants on the LDLR gene (NM_000527: c.C1783T: p.R595W, c.T493G: p.W165G, c.G1879A: p.A627T, c.G682T: p.E228X, and exon10: c.G1432A: p.G478R) and one pathogenic variant on APOB (NM_000384: c.C10579T: p.R3527W) in 25% of the identified definite FH patients. Two pathogenic mutations, c.T493G (p.W165G) and c.C1783T (p.R595W), were added to the current genetic spectrum of FH in China.

Conclusion: This study contributes to improving the current FH detection rate and genetic screening strategies; it provides new directions for treatment, management, and drug development.

KEYWORDS

familial hypercholesterolemia, genetic screening, *LDLR*, missense mutation, prevalence

1 | INTRODUCTION

Familial hypercholesterolemia (FH, OMIM#143890) is one of the most common autosomal-dominant genetic diseases. FH individuals exhibit high levels of low-density lipoprotein cholesterol (LDL-C), and some may be characterized by cutaneous xanthomas (Wang et al., 2018). If FH patients are not treated promptly, their risk of having coronary heart disease (CHD) rises to three to four times higher than those of patients with similarly elevated LDL levels but without FH diagnosis and the relative risk to the general population is even higher. Furthermore, they can have CHD before the age of 45, approximately 10 years earlier than non-FH patients (Jiang et al., 2015; Tomlinson et al., 2019). Moreover, FH has also been found to be responsible for >7% of premature myocardial infarction cases in China (Li et al., 2016). The prevalence of FH ranges from 1/200 to 1/500 in most populations (Xiang et al., 2017); the most recent prevalence of probable/definite FH in China was 0.35%, as estimated by the Dutch Lipid Clinical Network Criteria-Chinese Revised Version (DLCN-CRV) (Wang et al., 2019). However, FH is currently remarkably underdiagnosed in China. Limited prevalence data suggest that ~4.9 million people in China might be affected by FH, but only 513 FH cases have been reported in China as of 2019, including 70 homozygous, 91 compound heterozygous, 134 heterozygous, and 218 clinically diagnosed FH patients without genetic information (Mahdieh et al., 2020). This suggests that the majority of Chinese FH patients are undiagnosed and untreated due to the lack of awareness of FH among Chinese clinicians and the public (Pang et al., 2015). In addition, the limited availability of relevant data in China could be attributed to the lack of sufficient genetic testing and the absence of a national FH registry (Chen et al., 2019).

Therefore, to improve the current status of FH in China, it is an urgent need to increase the identification of FH cases and to expand the genetic spectrum of FH in China, to help clinicians better understand the interactions between mutations and LDL-C levels. This will also aid in increasing awareness of FH, leading to more effective screening and management of the disease in China.

Here, we applied a relatively stringent criterion (DLCN-CRV score ≥8) (Shi et al., 2014) on existing laboratory data of regular lipid profile tests and available clinical records in the hospital information system to identify clinically diagnosed definite FH patients. We then performed a laboratory-developed next-generation sequencing (NGS)-based genetic screening test on these patients to identify the responsible pathogenic mutations. The aims of this study were to roughly explore the prevalence of definite FH in the hospital-visiting population, determine the pathogenic mutation detection rate in clinically diagnosed FH patients, and expand the FH mutation spectrum in China.

2 | MATERIALS AND METHODS

2.1 | Subjects

The clinical records of 41,803 patients who had visited the First Hospital of China Medical University and whose blood lipid profiles had been tested during the period from November 11, 2019 to January 13, 2020 in the First Affiliated Hospital of China Medical University were investigated. In order to better identify the population with FH caused by genetic variations rather than other metabolic diseases, all patients with clinical diagnoses of diabetes, nephrotic syndrome, renal failure, pancreatitis, gout, alcoholism, kidney transplantation, bile duct blockage, hypertension, liver diseases, hypothyroidism, and other diseases that may cause secondary hyperlipidemia were excluded. Then, the DLCN-CRV (Shi et al., 2014) (Table S1) was applied to the remaining patients for the purpose of identifying FH patients. Finally, 18 patients were clinically diagnosed as definite FH patients (DLCN-CRV score ≥8). The general information of the 18 clinically diagnosed FH patients is shown in Table 2.

2.2 | Next-generation sequencing

Whole blood samples were collected from 18 (out of 173) definite FH patients who accepted the FH genetic screening test and 16 were finally tested for the FH genetic variants. Deoxyribonucleic acid (DNA) was extracted using the QIAamp Blood Midi Kit (Qiagen), following the official protocol provided in the user's manual. The DNA samples and libraries were then prepared according to

TABLE 1 Basic demographic characteristics of the 36,836 enrolled patients

	Definite FH	Probable FH	Possible FH	Unlikely FH
DLCN-CRV score	≥8 points	≥5 points	≥3 points	<3 points
Number (%)	173 (0.41%)	516 (1.23%)	7843 (18.76%)	28,304 (67.71%)
Female (%)	103 (59.54%)	306 (59.30%)	4024 (51.31%)	13,066 (46.16%)
Age (SD)	51.25 (13.58)	53.61 (13.73)	52.99 (13.56)	51.10 (16.42)
Prior MI (%)	4 (0.23%)	2 (0.39%)	7 (0.09%)	71 (0.25%)
On statin (%)	4 (0.23%)	8 (1.55%)	183 (2.33%)	2300 (8.13%)

Abbreviations: DLCN-CRV, Dutch Lipid Clinical Network Criteria-Chinese Revised Version; FH, familial hypercholesterolemia; MI, myocardial infarction.

TABLE 2 General information of patients with clinically diagnosed FH

No.	Gender	Age (years)	LDL-C (mM)	TC (mM)	HDL (mM)	TG (mM)	CAD/CVD	Treatment (daily)
01	Female	57	6.23	8.14	1.14	1.67	AMI[a]	No
02	Female	57	7.86	9.76	0.81	2.12		No
03	Female	55	6.59	8.74	1.51	0.94		No
04	Female	48	6.67	8.75	1.26	2.28	CHD[a]	No
05	Female	63	11.54	13.85	1.38	2.56		No
06	Female	62	8.53	10.27	0.84	1.75	CHD	Rosuvastatin 20 mg Ezetimibe 10 mg
07	Female	60	7.24	10.15	1.38	5.52		No
08	Male	49	6.56	8.61	1.14	1.57	CHD, AMI	Rosuvastatin 20 mg Ezetimibe 10 mg
09	Female	71	6.45	8.77	1.04	4.57	CHD	Rosuvastatin 20 mg Ezetimibe 10 mg
10	Female	72	7.62	11.84	3.31	0.8		No
11	Female	66	7.8	10.34	1.64	3.23		No
12	Male	50	8.1	10.25	0.92	2.83	CVD	Atorvastatin 20 mg Ezetimibe 10 mg
13	Male	51	6.44	8.16	0.91	0.84		No
14	Female	67	6.98	9.35	1.63	2.51		No
15	Female	59	6.63	8.96	1.43	1.5		No
16	Female	53	6.09	8.34	1.67	0.68		No
17	Male	46	6.78	8.56	1.42	1.59		No
18	Male	56	6.05	8.1	1.23	3.09		No
Reference interval				0–3.64	0–5.72	0.91–1.92	0–1.7	

Abbreviations: CAD, coronary artery disease; CHD, coronary heart disease; CVD, cerebrovascular disease; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride.

[a]The patient was just diagnosed for the first time.

the protocol of the 24-gene FH NGS screening test (24gFH NGS), which was designed to target the exon regions of 24 FH-related genes, as described in a previous study (Cheng et al., 2019). The 24 FH-related genes are described in the Table S2.

All these variants were analyzed using ANNOVAR (Wang et al., 2010) to obtain annotation information, including allele frequencies. Functional predictions were conducted using in silico predictive algorithms such as SIFT (Kumar et al., 2009), Polyphen2 (Adzhubei et al., 2010), MutationAssessor (Reva et al., 2007, 2011), and MutationTaster (Schwarz et al., 2014). The variants were then classified according to the 2010 American College of Medical Genetics and Genomics (ACMG) guidelines (Richards et al., 2015). If a novel variant was predicted to be pathogenic, damaging, not tolerated, or disease-causing by at least three of the four predictors, the variant was classified to be probably pathogenic

(class 4). If a variant could cause a frameshift, stop-codon introduction, splicing interruption, or had been previously reported as pathogenic, it was classified as pathogenic (class 5).

2.3 | Mutation validation

All identified class 4 or 5 variants were further verified using the ABI3730 Sanger sequencer (ABI, USA). The regions containing the targeted variants were sequenced using polymerase chain reaction (PCR) with the primers listed in the Table S3.

2.4 | Molecular structure modeling

The amino acid sequences of identified variants were entered into SWISS-MODEL (Waterhouse et al., 2018) to generate protein structure models; the structure of the extracellular domain of low-density lipoprotein receptor (LDLR; PDB ID: 1N7D.1) (Rudenko et al., 2002) was used as the template.

2.5 | Statistical methods

T tests were done by Microsoft Excel and applied for the comparison of the ages and LDL-C levels between different groups in this study.

3 | RESULTS

3.1 | FH prevalence and clinical features

During the 3-month period of retrospective investigation, 41,803 patients came to the first affiliated hospital of China Medical University and ordered lipid profile tests while seeing doctors or undergoing regular health checks. Among them, 173 (0.41% or 1 in 244) received definite diagnoses of FH according to the DLCN-CRV (score of ≥8) and 516 (1.23% or 1 in 81) were diagnosed as probable FH patients (DLCN-CRV score ≥5). The basic demographic characteristics of the 36,836 enrolled patients were listed in Table 1. One should note that this prevalence of 0.41% was only roughly estimated based on existing laboratory results considering that most patients did not provide enough information regarding their family history and DLCN-CRV did not include tendon xanthomas nor arcus in the criteria; therefore, the prevalence of definite FH reported in this study was surely underestimated.

The 173 definite FH patients were contacted to check whether they would come back for a genetic test for FH genetic variant screening purposes. Eighteen patients replied and agreed to the genetic test. The general information and clinical features of these 18 patients are described in Tables 1 and 2. Among them, 27.8% (5/18) were female and 72.2% (13/18) were male. The average age was 57.9 ± 7.8 years and the average level of LDL-C was 7.23 ± 1.30 mM (mean ± standard deviation). Three (16.7%) were previously diagnosed as CHD or acute myocardial infarction (AMI) patients, and one (5.56%) was previously diagnosed as cerebrovascular disease (CVD) patient. One (5.56%) was just diagnosed as a CHD patient and one (5.56%) was diagnosed as an AMI patient for the first time. Only the four patients with previous diagnoses of CHD/AMI/CVD had been regularly taking statins and ezetimibe. All others (77.8%, 14/18) were neither clinically treated nor informed of the possibility of having FH and high coronary artery disease (CAD) risks (including the two patients who had just been diagnosed as CHD and AMI patients). In addition, after the collection of their blood samples, two patients were excluded due to the unacceptable quality of the collected samples; therefore, 16 patients were finally included in the NGS test.

3.2 | Detection of candidate variants by NGS

The designed panel covered 98% of the exon region of the 24 genes (Table S2), including *LDLR* (OMIM:606945), *APOB* (OMIM:107730), *PCSK9* (OMIM:607786), *LDLRAP1* (OMIM:605747), *ABCG5* (OMIM:605459), and *ABCG8* (OMIM:605460). The in silico analyses indicated the existence of five pathogenic variants on the LDLR gene (NM_000527) and one pathogenic variant on APOB (NM_000384) in 25% of the clinically diagnosed patients (4/16): LDLR gene (NM_000527): exon12: c.C1783T: p.R595W, exon4: c.T493G: p.W165G, exon13: c.G1879A: p.A627T, exon4: c.G682T: p.E228X, exon10: c.G1432A: p.G478R, and APOB (NM_000384): exon26: c.C10579T: p.R3527W. Two patients were finally identified as compound and double heterozygous FH as they carried two pathogenic variants and their CAD risks were much higher than other heterozygous FH patients; therefore, they need to be treated with more intensive lipid-lowering therapy as soon as possible. The results of the in silico predictions and mutation validation tests are summarized in Table 3, together with the untreated cholesterol levels for the patients, respectively. Although large deletions or insertions cause around 5% of FH in Caucasian populations, we did not examine such insertions nor deletions for these 16 patients and this might underestimate the mutation

TABLE 3 Detected pathogenic variants

Gene	LDLR					APOB
Location	**NM_000527:exon12**	**NM_000527:exon13**	**NM_000527:exon4**	**NM_000527:exon10**	**NM_000527:exon4**	**NM_000384:exon26**
SNV	c.C1783T:p.R595W	c.G1879A:p.A627T	c.T493G:p.W165G	c.G1432A:p.G478R	c.G682T:p.E228X	c.C10579T:p.R3527W
Type	Missense SNV	Missense SNV	Missense SNV	Missense SNV	Stop-gain	Missense SNV
SIFT	0.0, deleterious	0.227, tolerated	0.001, deleterious	0.0, deleterious	–	0.0, deleterious
Polyphen2	1.0, damaging	0.972, damaging	1.0, damaging	1.0, damaging	–	1.0, damaging
MutationTaster	1, disease causing	1, disease causing	1, disease causing	1, disease causing	1, disease causing	0.998, disease causing
MutationAssessor	3.88, functional	1.96, functional	3.45, functional	3.925, functional	–	2.38, functional
Published	Yes, rs373371572	Yes, rs879255066	Yes, rs879254545	Yes, rs144614838	Yes, rs121908029	Yes, rs144467873
Patient no.	01	03	06	06	12	03
Genotype	C/T	G/A	T/G	G/A	G/T	C/T
ACMG class	4	4	4	4	5	4
LDL-C (mM)	6.23	6.59	8.53	8.53	8.1	6.59
TC (mM)	8.14	8.74	10.27	10.27	10.25	8.74
CAD/CVD	AMI[a]	–	CHD	CHD	CVD	–
Treatment	–	–	Rosuvastatin 20 mg Ezetimibe 10 mg	Rosuvastatin 20 mg Ezetimibe 10 mg	Atorvastatin 20 mg Ezetimibe 10 mg	–

Abbreviations: ACMG, American College of Medical Genetics; CAD, coronary artery disease; CHD, coronary heart disease; CVD, cerebrovascular disease; LDL-C, low-density lipoprotein cholesterol; LDL-R, low-density lipoprotein receptor; TC, total cholesterol.

[a]The patient was just diagnosed for the first time.

detection rate thus this should be considered as one of the limitations of this study.

In addition, one familial combined hypercholesterolemia pathogenic mutation (LPL(NM_000237): exon9: c.C1421G: p.S474X, ACMG Class 4) and one statin response-related genetic variant (SLCO1B1[NM_006446]: exon6: c.T521C: p.V174A) were also identified using the laboratory-developed test (LDT) protocol, as were two possible FH pathogenic mutations (ACMG Class 3, including LDLR(NM_000527): exon10: c.G1432A: p.G478R and exon7: c.1060+7T>C), and three variants (APOA5(NM_001166598): c.*158C>T, EPHX2(NM_001256483): exon7: c.G662A: p.R221Q, and USF1(NM_001276373): c.*187C>T) considered as risk factors for dyslipidemia. More variants that were identified by NGS and predicted to be relevant to hyperlipidemia are described in the Table S1. Although LDLR(NM_000527): exon7: c.1060+7T>C was identified as a possible FH pathogenic variant (ACMG Class 3), 11 out of 16 individuals were carriers of this variant so it appeared to be a common SNP in the Chinese population studied.

Moreover, three of the four patients (75%) that harbored the identified pathogenic variants were diagnosed as CHD/AMI/CVD patients, whereas only 21.4% of the remaining 14 patients that did not harbor pathogenic variants were diagnosed; the ages (58.4 ± 8.5 years and 56 ± 5.0 years, $p = 0.59$) and LDL-C levels (7.19 ± 1.38 mM and 7.36 ± 1.12 mM, $p = 0.82$) of these two groups were not significantly different from each other.

3.3 | Molecular structure model

The four pathogenic variants resided on exons 4, 12, and 13 of the LDLR gene. The variants c.T493G and c.G682T were located in the ligand binding region of the LDLR gene, and the variants c.C1783T and c.G1879A were located in the region of epidermal growth factor precursor homology (Figure 1).

The structure models were simulated using SWISS-MODEL. As shown in Figure 2, the p.E228X (c.G682T) model clearly demonstrated the loss of a portion of the protein structure compared with the wild-type model, with disruption of the conformation immediately before where the glutamic acid should appear. However, the other three models (c.T493G, c.C1783T, and c.G1879A) did not show any apparent structural change with respect to either the length of the protein or its tertiary structure (Figure 2).

As shown in Figure 3, the c.C1783T model showed that arginine in the position of the 595th amino acid was replaced by tryptophan. The c.G1879A model showed that alanine in the position of the 627th amino acid was replaced by threonine. The c.T493G model showed that tryptophan in the position of the 165th amino acid was replaced by glycine (Figure 3).

4 | DISCUSSION

To the best of our knowledge, this is the first retrospective study based on the laboratory results of biochemical assays to employ the DLCN-CRV to discover the prevalence of FH and the detection rate of FH mutations in clinically diagnosed FH patients (Shi et al., 2014). Four pathogenic LDLR variants were identified, including one variant reported in Asia for the first time, one variant reported in mainland China for the first time, and two variants belonging to the most commonly reported FH variants in Asia.

We found that the prevalence of definite FH was 0.41%, or 1 in 244, in the regular hospital-visiting population whose lipid profiles were tested, including inpatients, outpatients, and people, who only came to the hospital for regular health checks. The prevalence of the disease was indeed underestimated due to the lack of necessary information for outpatients and clinicians conducting health

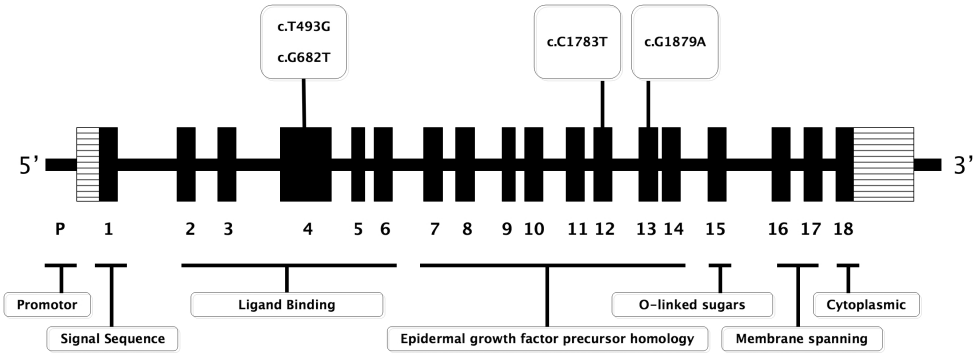

FIGURE 1 Diagram of the LDLR gene showing the four pathogenic variants identified in this study.

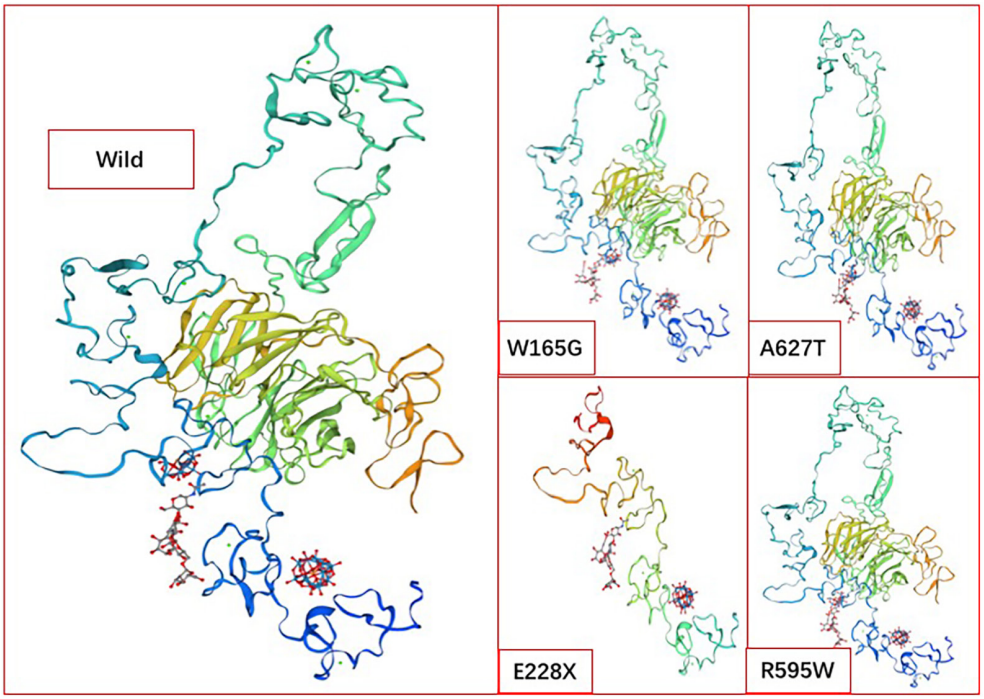

FIGURE 2 Simulated three-dimensional molecular structure models of the identified variant low-density lipoprotein receptor.

checks and due to the very stringent criteria applied (DLCN-CRV score ≥8). We applied such stringent criteria to deliberately increase the detection rate of pathogenic variants to discover those individuals who most require FH genetic tests, thus making FH genetic tests more cost-effective. In the 16 patients, who received genetic testing, we found that the mutation detection rate was 25% in individuals with DLCN-CRV scores of ≥8.

The FH prevalence reported in this study was similar to those reported in other populations, such as ~1 in 250 (0.4%) in general populations worldwide (Abul-Husn et al., 2016), 1 in 357 (0.35%) in the Chinese population (Shi et al., 2014), and roughly 7% in premature myocardial infarction patients (Li et al., 2016). The most important reason for this difference was that only definite FH cases with a DLCN-CRV score of 8 or higher were considered in this study, whereas FH cases with a DLCN-CRV score of 5 or higher were considered in other studies. However, considering the very large number of unidentified FH patients and the very low number of cases of FH reported in China, an FH identification rate of 0.41% seems appropriate because such retrospective investigations of the existing clinical records, using the laboratory results of lipid profile assays, caused no additional cost to patients nor clinicians. This study only investigated existing clinical records in a single hospital for a period of 3 months, yet still successfully identified 173 FH patients (33.72% of the total 513 reported FH cases in China as of 2019 (Mahdieh et al., 2020)), including four patients harboring FH pathogenic variants (2.26% of the 177 reported FH cases of pathogenic variants identified in China as of 2019 (Mahdieh et al., 2020)). This apparently increased the detection rate of FH in China, indicating that retrospective studies into existing clinical lipid profile records represent an effective way of identifying FH patients who are at high risk of CAD and can be treated to greatly improve their life quality and life expectancy.

Seventy-eight percent (14/18) of definite FH patients identified in this study were not informed nor treated prior to this study. The main reason was lipid profile that was not generally included in people's regular health checkup tests. Even if people received high LDL-C results in their previous health reports, they tended to consider this as a temporary condition due to a recent unhealthy diet rather than some disease requiring medical treatments. Moreover, none of the 18 FH patients knew that they had FH, even the six CAD/CVD patients and the four patients currently taking lipid-lowering medications. None of the treated patients met the LDL-C target. This indicates the urgent need to improve FH awareness in both patients and clinicians which would definitely increase the patients' compliance with undertaking LDL-C lowering treatments and further contribute to cardiovascular disease control in China.

Among the four FH pathogenic variants, the c.T493G (p.W165G) variant had never before been reported in Asia. It is located in the ligand binding region of the LDLR gene (Figure 1) and causes the tryptophan with a hydrophobic side chain in the position of the 165th amino acid to be replaced by glycine, with just hydrogen

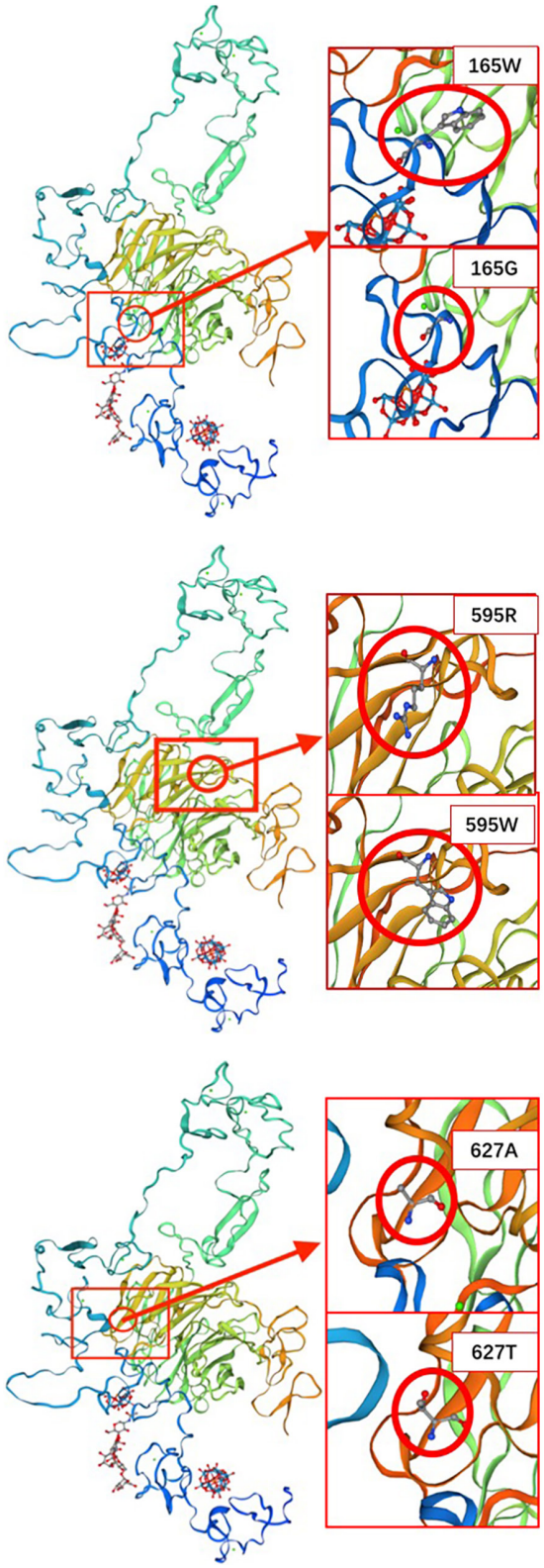

FIGURE 3 Simulated three-dimensional molecular structure model of the identified variant low-density lipoprotein receptor. The arrow indicates the position of the mutant amino acid and the molecular structural difference between the wild-type and mutant amino acids.

as a side chain (Figure 3). To date, this variant has only previously been reported in an Italian FH genetic mutation study in 2013 (Bertolini et al., 2013). Patient 06 in this study, who harbored c.T493G, was previously diagnosed with CVD and showed a high LDL-C level (8.53 mM), even after daily treatment with Rosuvastatin (20 mg) and Ezetimibe (10 mg). This indicates the urgent need to change to a more intensive lipid-lowering therapy (LLT).

The c.C1783T (p.R595W) variant was reported in this study for the first time in the Chinese mainland population. It is located in the epidermal growth factor precursor homology region of the LDLR gene (Figure 1) and causes arginine with a positively charged side chain in the position of the 595th amino acid to be replaced by tryptophan with a hydrophobic side chain (Figure 3). This variant has previously been found in two heterozygous FH patients in Taiwan (Chiou & Charng, 2010, 2012; Yang et al., 2007) and in one heterozygous FH patient in Singapore (Pek et al., 2018). Patient 01 in this study, who harbored c.C1783T, was only diagnosed with AMI on-site for the first time; they showed an LDL-C level of 6.23 mM without any medical treatment, which was lower than that of patient 06 but was much higher than the LDL-C target, indicating that they should immediately start intensive LLT.

C.G1879A (p.A627T) and c.G682T (p.E228X) are two common variants that have been reported by a number of studies in China, including Taiwan (Cao et al., 2018; Chang, 2003; Du et al., 2016; Jiang, Sun, et al., 2016; Jiang, Wu, et al., 2016; Mak et al., 1998). Furthermore, c.G682T has also been reported in other Asian countries, such as Korea (Han et al., 2015), Russia (Zakharova et al., 2005), and others (Reiman et al., 2016). C.G1879A is located in the epidermal growth factor precursor homology region of the LDLR gene (Figure 1); it causes alanine with a hydrophobic side chain in the position of the 627th amino acid to be replaced by threonine with a polar uncharged side chain (Figure 3). Patient 03 in this study, who harbored c.G1879A, showed an LDL-C level of 6.59 mM without any medical treatment; this was lower than those of patients 06 and 12 but was much higher than the LDL-C target, indicating that they should immediately start intensive LLT. C.G682T is located in the ligand binding region of the LDLR gene (Figure 1); it causes the loss of a portion of the protein structure compared with the wild-type model, with disruption of the conformation immediately before where glutamic acid should appear (Figure 2). Patient 12 in this study, who harbored c.T493G, was previously diagnosed with CVD and showed a high LDL-C level (8.1 mM) even after daily treatment with Atorvastatin (20 mg) and Ezetimibe (10 mg). This indicates the urgent need to change to a more intensive LLT.

Overall, 75% (3/4) of the four patients that harbored the identified pathogenic variants were diagnosed as CHD/AMI/CVD, whereas only 21.4% of the remaining 14 patients did not harbor pathogenic variants; the ages (58.4 ± 8.5 years and 56 ± 5.0 years, $p = 0.59$) and LDL-C levels (7.19 ± 1.38 mM and 7.36 ± 1.12 mM, $p = 0.82$) of these two groups were not significantly different from each other. This confirmed that FH mutation carriers have higher CAD risks than noncarriers with similar LDL-C levels, as previously reported by the JACC Scientific Expert Panel in 2018 (Sturm et al., 2018). Novel therapy options, such as PCSK9 inhibitors or lipid apheresis, are urgently needed for patients harboring FH mutations and those with high LDL-C levels, after treatment with the most intensive statin-based LLTs that such patients can tolerate.

In addition, one familial combined hypercholesterolemia pathogenic mutation (LPL(NM_000237): exon9: c.C1421G: p.S474X, ACMG Class 4) and one statin response-related genetic variant (SLCO1B1(NM_006446): exon6: c.T521C: p.V174A) were also identified using the LDT protocol, as were five possible FH pathogenic mutations (ACMG Class 3, including APOB(NM_000384): exon12: c.C1594T: p.R532W, exon26: c.C10579T: p.R3527W, exon29: c.G12809C: p.R4270T, and LDLR(NM_000527): exon10: c.G1432A: p.G478R, exon7: c.1060+7T>C), and three variants: (APOA5(NM_001166598): c.*158C>T, EPHX2(NM_001256483): exon7: c.G662A: p.R221Q, and USF1(NM_001276373): c.*187C>T) that were considered as risk factors for dyslipidemia. In this study, we did not examine the polygenic influence in FH patients, nor did we address mutations related to disorders other than FH. However, other variants that were identified by NGS and predicted to be relevant to hyperlipidemia are listed in the Table S1.

5 | CONCLUSION

In summary, we found that retrospectively investigating existing clinical records of laboratory lipid profile results using the DLCN-CRV greatly increased the detection rate of FH patients in China, at almost no additional cost. Eighteen definite FH cases, including two homozygous FH patients (one compound heterozygous and one double heterozygous), were identified and reported in this study. A 25% FH mutation detection rate and 6 pathogenic variants were obtained in patients with DLCN-CRV scores of ≥8, suggesting that this is a good criterion for clinicians to consider while ordering costly FH genetic tests for suspected FH patients. Furthermore, we have added two pathogenic mutations, c.T493G (p.W165G) and c.C1783T (p.R595W), to the current genetic spectrum of FH in China. Overall, these findings can contribute to improving current FH genetic screening strategies, and can help researchers to better understand genotype–phenotype correlations in FH. Further expansion of the genetic spectrum of FH could provide new directions for treatment, management, and drug development.

AUTHOR CONTRIBUTIONS

Jingxin Shan wrote the manuscript, worked on the study concept, and contributed to the data collection, experiments, and data analysis and interpretation. Shitong Cheng wrote the manuscript, supervised the whole study, and contributed to the study concept, results in analysis and interpretation, and manuscript review.

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CONFLICT OF INTEREST

The authors declared that they have no conflict of interest.

ETHICS STATEMENT

All individuals that underwent the genetic testing signed the informed consent form, and this study was reviewed and approved by the ethics committee of the First Hospital of China Medical University and conformed to the tenets of the Declaration of Helsinki.

DATA AVAILABILITY STATEMENT

Data available if one email the corresponding author and require.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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