

RESEARCH

Open Access



Familial hypercholesterolemia in Chinese children and adolescents: a multicenter study

Meng-Na Huang^{1†}, Chen-Cen Wang^{1,2†}, Ming-Sheng Ma^{3†}, Mei-Zhu Chi^{4†}, Qing Zhou^{5†}, Li-Hong Jiang^{6†}, Chun-Lin Wang^{7†}, Mei Lu^{8†}, Xiao-Quan Chen^{9†}, Ya-Ying Cheng^{10†}, Qing Ke^{11†}, Da-Yan Wang^{12†}, Xiao-Xia Qian^{13†}, Xiao-Ming Ying^{14†}, Jian-Ping Zhang^{15†}, Qun-Hua Shen^{16†}, Li-Fang Liu^{17†}, Rui Gu^{1,18}, Zhen-Jie Zhang³, Jian-Hua Feng⁹, Min Wang¹, Ming-Qiang Zhu¹, Ke Huang¹, Jun-Fen Fu^{1*} and Chao-Chun Zou^{1*}

Abstract

Background Familial hypercholesterolemia (FH) is an inherited disorder mainly marked by increased low-density lipoprotein cholesterol (LDL-C) concentrations and a heightened risk of early-onset arteriosclerotic cardiovascular disease (ASCVD). This study seeks to characterize the genetic spectrum and genotype–phenotype correlations of FH in Chinese pediatric individuals.

Methods Data were gathered from individuals diagnosed with FH either clinically or genetically at multiple hospitals across mainland China from January 2016 to June 2024.

Results In total, 140 children and adolescents (mean age of 6.00 years) with clinically and genetically diagnosed FH were enrolled in the study, with 87 distinct variants identified in the *LDLR*, *APOB* and *PCSK9* genes. Among the variants, 11 variants were newly identified worldwide, with 9 classified as “pathogenic” or “likely pathogenic”, and 2 classified as “variants of uncertain significance”. Additionally, the 5 most common variants in the study were c.1448G > A (p.W483*), c.1879G > A (p.A627T), c.1216C > A (p.R406R), and c.1747C > T (p.H583Y) in the *LDLR* gene, as well as c.10579C > T (p.R3527W) in the *APOB* gene, accounting for 49.29% (69/140) of all patients. These variants are primarily observed in the Asian or Chinese population and are distinct from those present in Caucasian groups. In this cohort, 105 patients were diagnosed with heterozygous FH (HeFH), while 35 were diagnosed with homozygous FH (HoFH). Finally, only 28.57% of the patients (40/140) were using lipid-lowering medications with 33.33% of HoFH patients initiating treatment after the age of 8. Additionally, only 3 compound heterozygous patients (2.14%) underwent liver transplantation because of significantly high lipid levels.

Conclusion This study reveals the variable genotypes and phenotypes of children with FH in China and illustrates that the genotypes in the Chinese population differ from those in Caucasians, providing a valuable dataset for the clinical genetic screening of FH in China. Furthermore, the older age at diagnosis and treatment highlights the underdiagnosis and undertreatment of Chinese FH pediatric patients, suggesting that early identification should

[†]Meng-Na Huang, Chen-Cen Wang, Ming-Sheng Ma, Mei-Zhu Chi, Qing Zhou, Li-Hong Jiang, Chun-Lin Wang, Mei Lu, Xiao-Quan Chen, Ya-Ying Cheng, Qing Ke, Da-Yan Wang, Xiao-Xia Qian, Xiao-Ming Ying, Jian-Ping Zhang, Qun-Hua Shen and Li-Fang Liu contributed equally to this work.

*Correspondence:

Jun-Fen Fu

fff68@zju.edu.cn

Chao-Chun Zou

zcc14@zju.edu.cn

Full list of author information is available at the end of the article



© The Author(s) 2024. **Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>.

be improved through lipid or genetic screening, and that more timely and regular pharmacological treatments should be implemented.

Keywords Familial hypercholesterolemia, Lipid, Low-density lipoprotein cholesterol, Genotype, Phenotype, Pediatrics

Introduction

Familial hypercholesterolemia (FH) is an autosomal inherited disorder resulting from pathogenic variants in the low-density lipoprotein (LDL) receptor (*LDLR*), its ligand apolipoprotein B (*APOB*), proprotein convertase subtilisin/kexin type 9 (*PCSK9*), and *LDLR* adaptor protein 1 (*LDLRAP1*) genes, which account for 85%–90%, 5%–10%, 1%–3%, and less than 1% of FH cases, respectively [1, 2]. In addition, more genes [e.g., growth hormone receptor (*GHR*), signal-transducing adaptor family member 1 (*STAP1*) and apolipoprotein E (*APOE*)] have been recognized as potential contributors to FH [3]. These variants impair the normal elimination of plasma LDL particles, thereby inducing significantly increased LDL cholesterol (LDL-C) concentrations over a lifetime and a considerable risk of early-onset ASCVD [4]. FH is clinically diagnosed based on phenotypic indicators such as high LDL-C levels, xanthomas, corneal arcus, early-onset ASCVD, and family history [5]. Furthermore, genetic testing is then recommended for clinically diagnosed patients or family cascade screenings of FH families [6].

FH is characterized by two forms: heterozygous HeFH) and homozygous (HoFH), depending on whether one or two pathogenic genes are present [1, 2]. HeFH is relatively common, occurring in about 1 in 311 to 500 people in the general population, whereas HoFH is extremely rare and severe, with an estimated incidence of 1 in 160,000–250,000 individuals [7–9]. Although the mutation spectrum and phenotype–genotype correlations of FH have been explored, about ninety percent of adults and ninety-five percent of children remain unrecognized, and FH patients often do not receive adequate treatment worldwide [10]. Owing to the large Chinese population, there may be 5.6 million HeFH patients and approximately 5,000 HoFH patients, with only 4.2% of confirmed or likely FH patients receiving lipid-lowering therapy (LLT) [11]. The underidentification and undertreatment of FH contribute to 10% of the population suffering from myocardial infarction before the age of 50, emphasizing the urgent need for early identification and treatment of FH [12]. Therefore, the clinical characteristics, genetic variants, and genotype–phenotype correlations in 140 FH pediatric patients and families in China were investigated between 2016 and 2024 to highlight the diagnosis and management of the orphan disease.

Materials and methods

Patients

A multicenter cohort study on pediatric patients (below the age of 18 years) diagnosed with FH both clinically and genetically was performed between January 2016 and June 2024. According to Chinese expert consensus, the enrollment criteria for the study were (1) fasting pre-treatment LDL-C concentration >4.90 mmol/L (190 mg/dL) on the basis of two consecutive measurements; (2) fasting pre-treatment LDL-C concentration >3.60 mmol/L (140 mg/dL) with xanthomas, corneal arcus, or premature ASCVD; (3) fasting pre-treatment LDL-C concentration >3.60 mmol/L (140 mg/dL) with a familial history of hypercholesterolemia and/or premature ASCVD in first- or second-degree relatives; and (4) the presence of one or two pathogenic or likely pathogenic variants in the *LDLR*, *APOB*, or *PCSK9* genes or two pathogenic or likely pathogenic variants in the *LDLRAP1* gene through genetic testing [13]. Additionally, HoFH should be diagnosed clinically when the fasting pre-treatment LDL-C concentration is ≥ 13 mmol/L (500 mg/dL).

Additionally, patients with combined hyperlipidemia, hypertriglyceridemia, and other conditions that could induce secondary hypercholesterolemia (e.g., Cushing's syndrome, nephrotic syndrome, Wilson's disease and other definitive primary liver or kidney diseases) were excluded. The research participants consisted of individuals genetically diagnosed with HeFH and HoFH.

Clinical characteristics and laboratory investigations

Clinical data, including demographics (age and sex), clinical features (e.g., xanthomas and corneal arcus), height (m), weight (kg), physical examination findings, and personal and familial histories of dyslipidemia and ASCVD, were collected at the time of initial diagnosis. Onset age is defined as the age when xanthomas or elevated lipid levels were first detected. Diagnostic age is defined as the age when the patient was genetically diagnosed with FH. Inclusion age is defined as the age when the patient was enrolled in the study.

Total cholesterol (TC), LDL-C, high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), apolipoprotein A (ApoA), ApoB, and lipoprotein (a) [Lp(a)] were evaluated in blood samples collected in the morning following an overnight fast. The patients also underwent Doppler echocardiography to evaluate the anatomy of the

carotid artery and heart, especially the carotid intima-media thickness (cIMT). Based on medical records and follow-up phone calls, lipid-lowering therapy data were also collected from the participants.

Genetic analysis

Patients and their first-degree relatives provided peripheral blood samples for genomic DNA extraction. The DNA was fragmented by sonication, followed by end repair and adapter attachment at both ends. After amplifying the library, the DNA fragments were captured and enriched via an exon chip and then further amplified and subjected to WES via the Illumina platform. Sanger sequencing was used to confirm the identified variant in other family members for certain patients. The human genome reference sequence hg19 was used to align the obtained DNA sequences via the Barrows–Wheeler Aligner (BWA), in order to assess the coverage and quality of sequencing in the target regions. The Guangzhou KingMed Center for Clinical Laboratory conducted the analysis.

The Leiden Open Variation Database (LOVD3), the Human Gene Mutation Database (HGMD), and ClinVar were used to check all identified variants for novelty. Predictions regarding protein structure and functional alterations were evaluated by Sorting Intolerant From Tolerant (SIFT), Polymorphism Phenotyping v2 (PolyPhen-2), and Mutation Taster.

In accordance with the recommendations of the American College of Medical Genetics (ACMG) and the classification guidelines modified by the Clinical Genome Resource (ClinGen) Familial Hypercholesterolemia Variant Curation Expert Panel [14, 15], the pathogenicity of the variants was assessed and classified as pathogenic (P), likely pathogenic (LP), variants of uncertain significance (VUS), likely benign (LB), or benign (B). Additionally, the Human Genome Variation Society (HGVS) provided guidelines for naming diverse variants. LDLR variants have been classified into “null” and “defective” categories depending on functional changes. Truncating variants, altered translation initiation sites, frameshift mutations, splice-site alterations, and large genomic rearrangements involving one or more exons are all types of “null” variants in LDLR, which lead to the production of either a completely defective or absent protein. In contrast, “defective” variants refer to missense variants that cause partial loss of function (LOF) or altered function of the receptor [8]. Given that various variant types and statuses can affect protein function differently, heterozygous patients were categorized into three groups: HeFH with APOB/PCSK9 variants, HeFH with defective LDLR variants, and HeFH with null LDLR variants. In addition, homozygous FH strains were categorized into double

heterozygotes (two variants in 2 different genes), compound heterozygotes (two variants in 2 different alleles of the same gene) or true homozygotes (two variants in the same allele) [16].

Statistical analysis

SPSS software (version 26.0 for Mac; SPSS Inc., Chicago, IL, USA) was used to perform the statistical analysis. Data with a normal distribution are expressed as means \pm standard deviations (means \pm SDs) and were analyzed using Student's t-test or one-way ANOVA. Data with a nonnormal distribution are expressed as the medians with interquartile ranges [medians, (IQRs)] and were analyzed using the Mann–Whitney or Kruskal–Wallis test. The frequencies (%) of categorical variables were reported and assessed with the Chi-square test or Fisher's exact method. In addition, multivariate logistic regression and analysis of covariance (ANCOVA) were performed to adjust for significant differences in the age at initial diagnosis. Graphics were created via GraphPad Prism 10 and MS Excel 2021. Statistical significance was deemed to be present when the *P* value was below 0.05.

Results

Study population

A total of 170 clinically diagnosed subjects were recruited over the past eight years. Through molecular testing, 26 individuals were diagnosed with variants in ATP-binding cassette subfamily G member 5 (*ABCG5*) and ATP-binding cassette subfamily G member 8 (*ABCG8*) was subsequently excluded from the study [17]. In addition, 4 individuals with no detectable variants were also excluded. Overall, 140 patients with identified positive variants participated in the study. This cohort consisted of patients with increased LDL-C levels and pathogenic or likely pathogenic variants, as well as patients with VUS or likely benign variants, increased LDL-C concentrations and a relevant familial history of hypercholesterolemia.

Among them, 88.57% ($n=124$) were from southern China, with the majority of families coming from Zhejiang Province ($n=106$, 75.70%), while the second-largest group was from the Beijing-Tianjin-Hebei region ($n=12$, 8.57%).

Baseline characteristics

The baseline characteristics of the cohort ($n=140$, 52.14% male) are presented in Table 1. The majority of patients ($n=101$, 72.14%) visited the outpatient clinic for the first time because of elevated lipid levels discovered incidentally during laboratory investigations. Nevertheless, 29 patients (20.71%) were initially detected due to the presence of xanthomas, and 5 of them underwent

Table 1 The baseline characteristics of all patients ($n=140$)

Items		All patients
Patients		140
Male, n (%)		73 (52.14%)
Age at onset (year)		5.00 (2.50, 8.58)
Age at diagnosis (year)		6.00 (3.33, 9.00)
Age at inclusion (year)		8.25 (5.50, 11.75)
Weight (kg)		19.15 (15.50, 26.50)
Weight SDS		-0.40 (-0.88, 0.70)
Height (m)		1.16 ± 0.03
Height SDS		-0.23 ± 0.11
BMI (kg/m ²)		16.02 ± 0.32
BMI SDS		-0.17 ± 0.15
Xanthomas (%)		36 (25.71%)
Corneal arcus (%)		6 (4.28%)
Increased cIMT, (%)		10 (7.14%)
Family history of HC, n (%)	first-degree	125 (89.29%)
	second-degree	73 (52.14%)
Family history of ASCVD, n (%)	first-degree	7 (5.00%)
	second-degree	17 (12.14%)
Lipid levels	TC, mmol/L	8.35 (7.46, 11.27)
	TG, mmol/L	0.98 (0.72, 1.35)
	LDL-C, mmol/L	6.08 (5.15, 8.97)
	HDL-C, mmol/L	1.40 (1.17, 1.71)
	LDL/HDL	4.47 (3.46, 5.80)
	APOA, mg/d L	122.94 ± 4.76
	APOB, mg/d L	153.35 (129.65, 216.50)
	APOB/APOA	1.42 (0.94, 1.91)
	Lp(a), mg/d L	18.90 (12.10, 26.20)
Lipid-lowering therapy	Low-lipid diet, n (%)	97 (69.29%)
	Drugs, n (%)	40 (28.57%)
	Liver translation, n (%)	3 (2.14%)

BMI body mass index, **SDS** standard deviation score, **cIMT** carotid intima-media thickness, **n** number, **HC** Hypercholesterolemia, **ASCVD** arteriosclerotic cardiovascular disease, **TC** total cholesterol, **TG** triglyceride, **LDL-C** low-density lipoprotein cholesterol, **HDL-C** high-density lipoprotein cholesterol, **APOA** apolipoprotein A, **APOB** apolipoprotein B, **Lp(a)**, lipoprotein (a). Normally distributed data are presented as the means with standard deviations (means ± SD) and nonnormally distributed data are reported as medians with interquartile ranges [medians, (IQRs)]

skin biopsies to confirm the diagnosis, which resulted in a delay in their diagnosis. Additionally, 10 patients (7.14%) were detected through genetic testing of family members. The median age at the time of onset was 5.00 years, with the range spanning from 0.08 to 16.50 years. The diagnosis was established at an average age of 6.00 years, ranging between 0.17 and 17.00 years. Moreover, only 40.71% (57/140) of patients received an FH diagnosis before the age of 5. Among these patients, the prevalence rates of xanthoma, corneal arcus, and thickening cIMT were 25.71%, 4.28%, and 7.14%, respectively. Four individuals developed atherosclerotic deposits in the carotid arteries.

The average levels of fasting untreated lipid profiles are also presented in Table 1. Notably, LDL-C concentrations ranging from 3.6 mmol/L to 4.9 mmol/L were observed in 15.00% (21/140) of patients, which do not meet the recommended cutoff values for FH as outlined in the American Multisociety 2018 lipid guidelines [18]. At the time of inclusion in this study, 69.28% (97/140) of patients were following a low-lipid diet to lower their lipid levels, 28.57% (40/140) were using lipid-lowering medications, with the majority taking statins and/or ezetimibe, and only 3 compound heterozygous patients (2.14%) underwent liver transplantation owing to significantly high lipid levels.

Molecular analysis

Genetic testing was conducted for all patients. In total, 140 patients with one or two positive variants in *LDLR*, *APOB* or *PCSK9* were identified. Of these, 75.00% ($n=105$) were heterozygous carriers, consisting of 93 individuals with *LDLR* variants, 11 with *APOB* variants, and 1 with a *PCSK9* variant. The remaining 25.00% ($n=35$) were homozygous, including 27 compound heterozygotes, 5 true homozygotes for *LDLR*, and 3 individuals with double heterozygous variants. Details of these identified homozygous genotypes are provided in Supplementary Table 1.

Additionally, 87 different variants were identified, which are presented in Supplementary Table 2. Figure 1 illustrates the distribution of the identified variants. Specifically, of the 87 distinct variants, 80 were in the *LDLR* gene, accounting for 91.95%, including 39 missense variants, 11 nonsense variants, 1 synonymous variant, 13 frameshift variants, 13 splice site variants, 1 in-frame deletion, and 2 exon deletions. The *APOB* and *PCSK9* variants accounted for 6.90% (6/87) and 1.15% (1/87), respectively. According to the ACMG and ClinGen classifications, 86.21% ($n=75$) of the variants identified were categorized as pathogenic or likely pathogenic, 14.94% ($n=13$) were classified as VUS, and 1.15% ($n=1$) were classified as likely benign. To analyze the pathogenicity of 13 VUSs and 1 likely benign variant, in silico analysis, in vitro functional studies and family analysis of these variants are shown in Table 2 [19, 20]. Furthermore, the clinical characteristics of patients with VUS or likely benign variants were compared with those of patients with pathogenic or likely pathogenic variants. The analysis found no significant variation in lipid profiles across these groups, as detailed in Supplementary Table 3.

Among the 87 variants, 11 (12.64%), including 6 frameshift variants (54.55%), 2 missense variants (18.18%), 2 exon deletions (18.18%), and 1 nonsense variant (9.09%), had not been previously reported worldwide. The key characteristics of these novel variants are

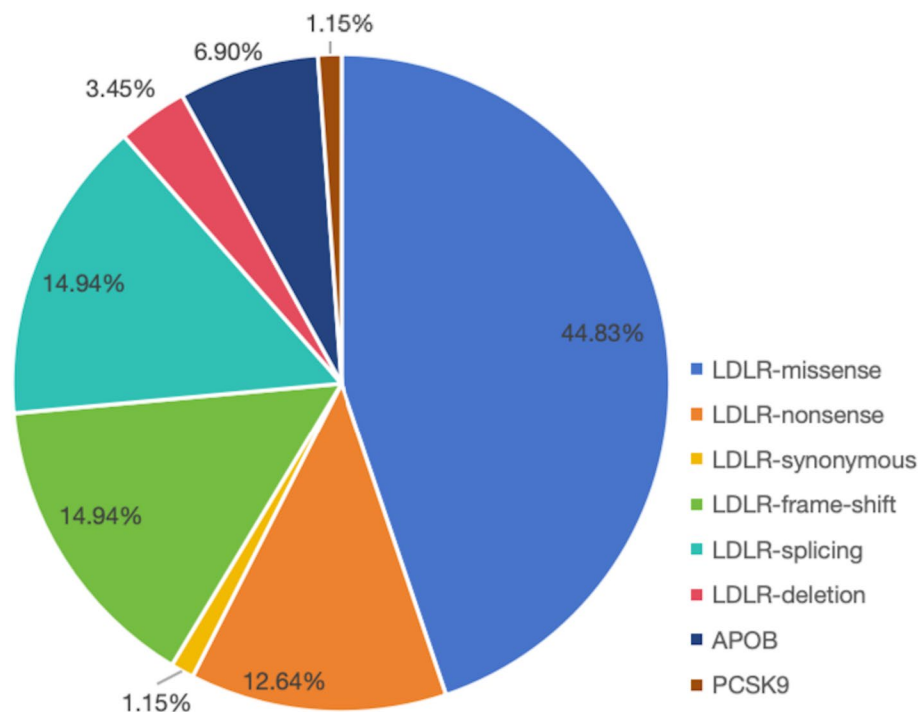


Fig. 1 The genotypes of familial hypercholesterolemia ($n=87$) identified in the present study. FH: familial hypercholesterolemia; LDLR: low-density lipoprotein receptor; APOB: apolipoprotein B; PCSK9: proprotein convertase subtilisin/Kexin type 9

presented in Table 3. In accordance with the ACMG and ClinGen classifications, all novel variants were assessed for pathogenicity, and 81.82% of them (9/11) were categorized as pathogenic or likely pathogenic, whereas 2 missense variants (18.18%) were classified as VUS. The analysis found no significant variation in lipid profiles across patients with novel variants and those with known variants, as shown in Supplementary Table 4. In addition, 16 variants were found in more than one individual, and the 5 most common variants were c.1448G>A (p.W483*, 13.57%), c.1879G>A (p.A627T, 10.00%), c.1747C>T (p.H583Y, 10.00%), and c.1216C>A (p.R406R, 9.29%) of the *LDLR* gene and c.10579C>T (p.R3527W, 6.43%) of the *APOB* gene, accounting for 49.29% (69/140) of all patients. The *LDLR* variants were distributed across 18 exons and 6 introns, with the most frequent variants occurring between exon 3 (10.00%, 8/80) and exon 4 (20.00%, 16/80), which encode the *LDLR* ligand-binding domain (Fig. 2). The most common *APOB* variant identified in these patients was c.10579C>T (64.29%, 9/14), which is located in exon 26.

Genotype–phenotype correlation

The comparison of the clinical characteristics of HeFH and HoFH patients was made to correlate genotype and phenotype, as presented in Table 4. HoFH patients exhibited a more severe phenotype, with significantly greater

lipid profiles levels than HeFH patients did ($P<0.05$ for all), except for TG and HDL-C levels. Furthermore, the LDL-C levels of most HeFH patients (63.81%, 67/105) were between 3.6 mmol/L and 4.9 mmol/L, whereas the LDL-C levels of most HoFH patients (60.00%, 21/35) exceeded 13 mmol/L (Fig. 3A). Given the certain degree of overlap in LDL-C levels between the two genotypes, the LDL-C cutoff value for distinguishing HoFH patients from HeFH patients was determined through receiver operating characteristic (ROC) curve analysis (Fig. 3B). The analysis revealed a cutoff value of 10.34 mmol/L, which resulted in a sensitivity of 79.4%, a specificity of 99.0%, and an AUC of 0.893 ($P<0.001$).

In addition, individuals with more than one genetic variant causing FH were diagnosed at a younger age (4.00 in HoFH vs. 7.00 in HeFH) and presented a higher incidence of xanthomas, corneal arcus, and increased cIMT ($P<0.05$). The primary reason for the initial diagnosis of HeFH was the incidental finding of elevated blood lipids during a routine check-up (88.57%, 93/105), whereas the main reason for the initial diagnosis of HoFH was the presence of disseminated xanthomas (74.29%, 26/35). For lipid-lowering therapy, a low-lipid diet alone was utilized more frequently by HeFH patients compared to HoFH patients (84.76% in HeFH patients vs. 22.85% in HoFH patients, $P<0.001$), whereas lipid-lowering medication use was

Table 2 Pathogenicity prediction of VUS and LB variants identified in the cohort with FH

Gene	Location	Variants	Type of variant	Number of patients (%)	Novelty	Variant rating of ACMG and ClinGen	In silico analysis		In vitro function study	Family analysis	
							Polyphen-2	SIFT2			Mutation Taster
LDLR	exon3	c.233G>A (p.R78H)	Missense	1 (0.71%)	Known	VUS	Most likely, damaging	Deleterious	Disease causing	n/a	Relatives with the same variant had hypercholesterolemia
LDLR	exon3	c.292G>A (p.G98S)	Missense	1 (0.71%)	Known	VUS	Most likely, damaging	Deleterious	Disease causing	No effect (Jiang L et al. Atherosclerosis.2017;263:163–170.)	Father with same variant had hypercholesterolemia
LDLR	exon5	c.769C>T (p.R257W)	Missense	3 (2.14%)	Known	VUS	Most likely, damaging	Deleterious	Polymorphism	No adverse effect (Jiang L et al. Atherosclerosis.2017;263:163–170.)	Relatives with the same variant had hypercholesterolemia
LDLR	exon6	c.895G>T (p.A299S)	Missense	1 (0.71%)	Known	VUS	Benign	Tolerated	Polymorphism	n/a	Relatives with the same variant had hypercholesterolemia
LDLR	exon7	c.1060+10G>A	Splicing	1 (0.71%)	Known	VUS	n/a	n/a	n/a	n/a	Relatives with the same variant had hypercholesterolemia
LDLR	exon9	c.1277T>C (p.L426P)	Missense	1 (0.71%)	Known	VUS	Benign	Tolerated	Disease causing	n/a	Relatives with the same variant had hypercholesterolemia
LDLR	exon11	c.1636G>A (p.G546S)	Missense	1 (0.71%)	New	VUS	Most likely, damaging	Deleterious	Disease causing	n/a	Father with same variant had hypercholesterolemia
LDLR	exon12	c.1721G>T (p.R574L)	Missense	1 (0.71%)	Known	VUS	Most likely, damaging	Deleterious	Disease causing	n/a	Mother with same variant had hypercholesterolemia
LDLR	exon13	c.1975A>C (p.T659P)	Missense	1 (0.71%)	Known	LB	Benign	Tolerated	Polymorphism	n/a	Relatives with the same variant had hypercholesterolemia
LDLR	exon13	c.1885G>A (p.V797M)	Missense	1 (0.71%)	New	VUS	Benign	Tolerated	Disease causing	n/a	Relatives with the same variant had hypercholesterolemia
LDLR	exon16	c.2344A>C (p.K782Q)	Missense	1 (0.71%)	Known	VUS	Benign	Tolerated	Polymorphism	n/a	Relatives with the same variant had hypercholesterolemia

Table 2 (continued)

Gene	Location	Variants	Type of variant	Number of patients (%)	Novelty	Variant rating of ACMG and ClinGen	In silico analysis			In vitro function study	Family analysis
							Polyphen-2	SIFT2	Mutation Taster		
APOB	exon17	c.2563G>T (p.A855S)	Missense	1 (0.71%)	Known	VUS	Most likely, damaging	Tolerated	Polymorphism	n/a	Father with same variant had hypercholesterolemia
APOB	exon29	c.12581 T>C (p.I4194T)	Missense	1 (0.71%)	Known	VUS	Benign	Tolerated	Polymorphism	n/a	Mother with same variant had hypercholesterolemia
APOB	intron1	c.64_66dup (p.L22dup)	Ins/del	1 (0.71%)	Known	VUS	n/a	Tolerated	Polymorphism	n/a	Father with same variant had hypercholesterolemia

n/a not available, P pathogenic, LP likely pathogenic, VUS variants of uncertain significance, LB likely benign

Table 3 Description and function prediction of globally identified novel variants in the cohort with FH

Gene	Transcript	Location	Sequence variant	Predicted effect	Polyphen-2	SIFT2	Mutation Taster	Type of variant	Number of patients (%)	Variant rating of ACMG and ClinGen
LDLR	NM_000527.5	exon4	c.497delCinsGGA TCCCCCAGCTGC TACCCCCAG	p.A166Gfs*48	n/a	n/a	Disease causing	Frameshift	1 (0.71%)	P
LDLR	NM_000527.5	exon5	c.722delT	p.F241Sfs*24	n/a	n/a	Disease causing	Frameshift	1 (0.71%)	LP
LDLR	NM_000527.5	exon6	c.880delA	p.K294Kfs*76	n/a	n/a	Disease causing	Frameshift	1 (0.71%)	LP
LDLR	NM_000527.5	exon7	c.1009G>T	p.E337*	n/a	n/a	Disease causing	Nonsense	1 (0.71%)	P
LDLR	NM_000527.5	exon9	c.1206delC	p.F403Sfs*10	n/a	n/a	Disease causing	Frameshift	1 (0.71%)	P
LDLR	NM_000527.5	exon11	c.1636G>A	p.G546S	Most likely, damaging	Deleterious	Disease causing	Missense	1 (0.71%)	VUS
LDLR	NM_000527.5	exon11	c.1637dup	p.L547Pfs*12	n/a	n/a	Disease causing	Frameshift	1 (0.71%)	P
LDLR	NM_000527.5	exon12	c.1747_1748delCA	p.H583Lfs*19	n/a	n/a	Disease causing	Frameshift	1 (0.71%)	P
LDLR	NM_000527.5	exon13	c.1885G>A	p.V797M	Benign	Tolerated	Disease causing	Missense	1 (0.71%)	VUS
LDLR	NM_000527.5	exon16-18	exon16-18 deletion	n/a	n/a	n/a	n/a	Deletion	2 (1.43%)	P
APOB	NM_000384.3	exon24-29	exon24-29 deletion	n/a	n/a	n/a	n/a	Deletion	1 (0.71%)	LP

n/a not available, P pathogenic, LP likely pathogenic, VUS variants of uncertain significance, LB likely benign

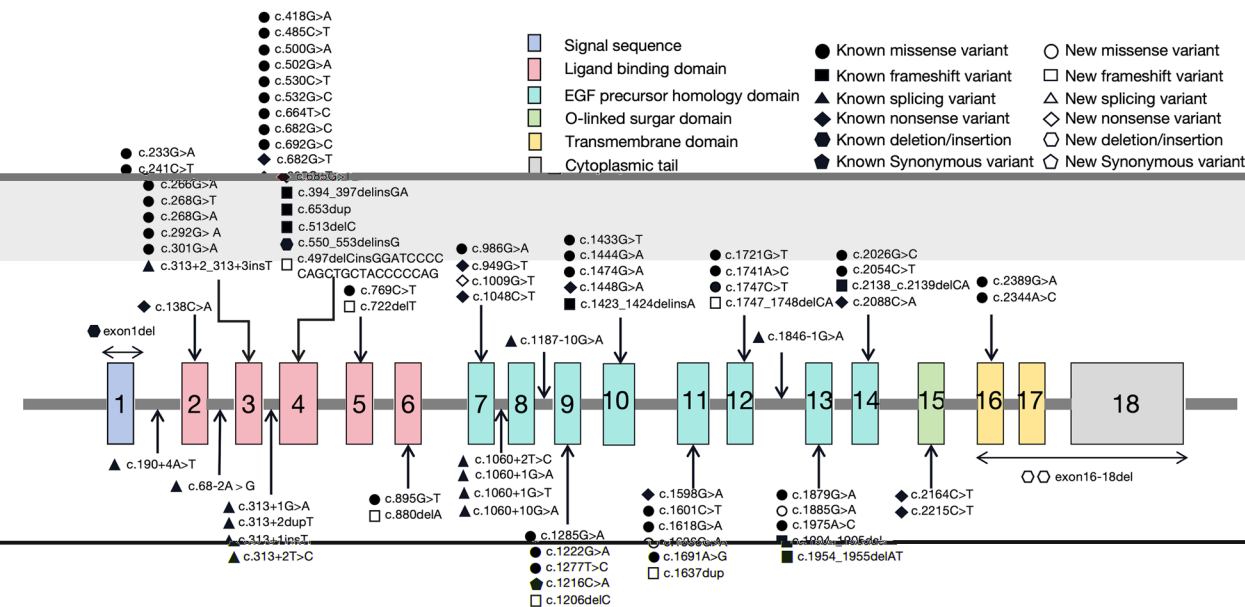


Fig. 2 Positions of the variants identified in the *LDLR* gene. Schematic representation of *LDLR* exons (exons of different colors encode different functional protein domains) with the relative locations of the detected variants (different shapes represent different kinds of variants)

found to be statistically more common in the homozygous group (68.57% vs. 15.23% in HeFH patients, $P < 0.001$) and liver transplantation (8.57% vs. 0.00% in HeFH patients, $P = 0.015$) compared to HeFH patients (Table 4). Furthermore, HeFH patients started using lipid-lowering drugs at an older median age compared to HoFH patients (11.71 years for HeFH vs. 6.46 years for HoFH, $P < 0.001$). Notably, 75.00% of HeFH patients began using lipid-lowering medications after the age of 8, whereas 66.67% of HoFH patients started before the age of 8.

On the basis of the functional changes in *LDLR*, the variants were classified into "null" variants and "defective" variants. Compared with those with *LDLR*-defective variants or *APOB/PCSK9* variants, heterozygotes with *LDLR*-null variants presented higher LDL-C levels (5.81 vs. 5.24 vs. 5.80, respectively; $P = 0.004$) and LDL-C/HDL-C ratios (4.14 vs. 3.96 vs. 2.88, respectively; $P = 0.042$) (Table 5).

With respect to the homozygous genotype, the 3 patients with double heterozygotes presented a considerably milder phenotype, with markedly lower levels of lipid profiles and *APOB* than did those with compound heterozygotes and true homozygotes ($P < 0.05$ for all) (Table 6). Furthermore, the prevalence of xanthomas in double heterozygotes was substantially lower than that in compound heterozygotes and true homozygotes (0% vs. 92.6% vs. 100%, $P = 0.002$). In terms of carotid artery ultrasound analyses, 60.00% of patients ($n = 3$) with true homozygotes had increased cIMT, which was

greater than the 33.33% of patients ($n = 1$) with double heterozygotes and the 7.41% of patients ($n = 2$) with compound heterozygotes ($P = 0.018$). Moreover, Fig. 4 illustrates the average LDL-C levels in patients with different heterozygous and homozygous FH types.

Finally, the clinical characteristics were compared between HeFH patients carrying the 4 most frequent variants (c.1747C > T, c.1448G > A, c.1216C > A in *LDLR*, and c.10579C > T in *APOB*) and those with other variants is presented in Table 7. Although individuals with the c.10579C > T variant presented relatively high concentrations of elevated TC and LDL-C, no statistically meaningful differences were observed ($P > 0.05$). Moreover, the LDL-C level variability among subjects with the same variant was assessed. As shown in the violin plots in Fig. 5, the c.10579C > T variant exhibited the broadest range of LDL-C values (a maximum-to-minimum variation of 5.10 mmol/L), while the c.1747C > T variant displayed the lowest variability in LDL-C levels (a maximum-to-minimum variation of 2.67 mmol/L).

Discussion

This study details the clinical presentation, biochemical markers and genetic data of 140 pediatric subjects with HeFH and HoFH from multiple centers in China. As far as we are aware, this is the most sizable cohort identified up to the present that outlines the genotype and genotype-phenotype associations in the pediatric population of China.

Table 4 The clinical characteristics of HeFH patients and HoFH patients ($n = 140$)

	HeFH	HoFH	t/ χ^2	P value
Patients	105	35		
Male, n (%)	55 (52.38%)	18 (51.43%)	0.01	1
Age at onset (year)	6.00 (3.55, 9.00)	2.50 (1.50, 4.79)	-4.657	< 0.001
Age at diagnosis (year)	7.00 (4.13, 9.59)	4.00 (2.21, 6.63)	-3.521	< 0.001
Age at inclusion (year)	9.33 (5.96, 11.83)	6.75 (4.29, 11.00)	-1.441	0.149
BMI (kg/m^2)	15.59 (14.58, 17.29)	15.11 (14.49, 15.76)	0.067	0.947
BMI SDS	-0.14 \pm 0.18	-0.25 \pm 0.26	0.346	0.730
Xanthomas (%)	6 (5.71%)	30 (85.71%)	45.267	< 0.001
Corneal arcus (%)	0	6 (17.14%)		< 0.001
Increased cIMT, (%)	4 (3.81%)	6 (17.14%)	6.927	0.008
First-degree family history of HC, n (%)	91 (86.66%)	34 (97.14%)	3.037	0.081
Second-degree family history of HC, n (%)	53 (50.47%)	20 (57.14%)	0.393	0.531
First-degree family history of ASCVD, n (%)	6 (5.71%)	1 (2.85%)	0.277	0.598
Second-degree family history of ASCVD, n (%)	11 (10.47%)	6 (17.14%)	1.503	0.22
TC, mmol/L	7.95 (7.30, 8.76)	17.81 (12.88, 21.30)	11.744	< 0.001
TG, mmol/L	0.99 (0.70, 1.29)	0.97 (0.83, 1.39)	-0.178	0.859
LDL-C, mmol/L	5.65 (5.05, 6.39)	14.36 (10.66, 19.03)	12.195	< 0.001
HDL-C, mmol/L	1.44 (1.22, 1.71)	1.28 (0.98, 1.69)	-0.466	0.642
LDL/HDL	3.75 (3.17, 4.79)	9.83 (5.05, 16.81)	6.426	< 0.001
APOA, mg/dl	129.50 \pm 5.82	104.12 \pm 5.54	2.434	0.018
APOB, mg/dl	143.60 (128.00, 161.70)	305.00 (199.00, 333.00)	-4.251	< 0.001
APOB/APOA	1.10 (0.86, 1.53)	2.94 (1.91, 3.71)	-4.329	< 0.001
Lp(a), mg/dl	15.90 (11.06, 24.93)	20.65 (18.33, 51.15)	1.057	0.296
low-lipid diet, n (%)	89 (84.76%)	8 (22.85%)	29.944	< 0.001
Lipid-lowering drugs, n (%)	16 (15.23%)	24 (68.57%)	26.146	< 0.001
Liver transplantation, n (%)	0	3 (8.57%)		0.015

BMI body mass index, SDS standard deviation score, cIMT carotid intima-media thickness, n number, HC Hypercholesterolemia, ASCVD arteriosclerotic cardiovascular disease, TC total cholesterol, TG triglyceride, LDL-C low-density lipoprotein cholesterol, HDL-C high-density lipoprotein cholesterol, APOA apolipoprotein A, APOB apolipoprotein B, Lp(a) lipoprotein (a). Normally distributed data are presented as means with standard deviations (means \pm SD) and were analyzed using Student's t -test, with the corresponding t and P values. Non-normally distributed data are presented as medians with interquartile ranges [medians, (IQRs)] and were compared using the Mann-Whitney test, with the corresponding U and P values. Categorical data are expressed as frequencies (%) and were assessed using the χ^2 test or Fisher's exact test, with the corresponding χ^2 and P values.

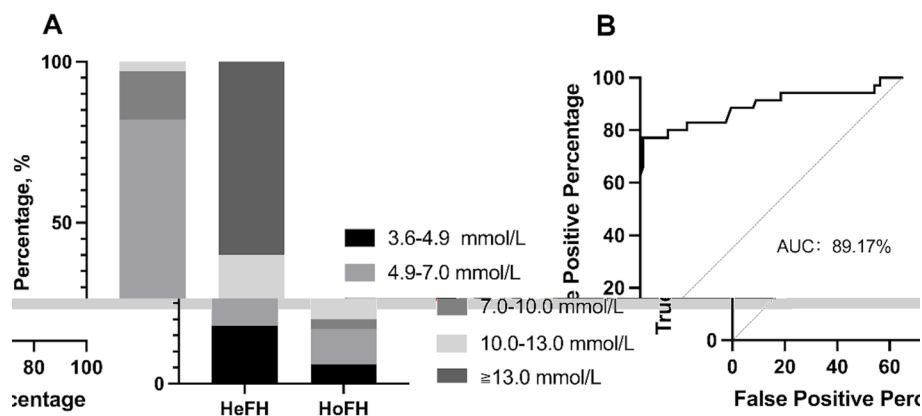


Fig. 3 Low-density lipoprotein-cholesterol (LDL-C) levels in HeFH patients and HoFH patients. **A** Frequencies of LDL-C subgroups in HeFH patients and HoFH patients. **B** Receiver operating characteristic (ROC) curves evaluating the ability of low-density lipoprotein (LDL)-cholesterol values to distinguish between HeFH patients and HoFH patients. HeFH, heterozygous familial hypercholesterolemia; HoFH, homozygous familial hypercholesterolemia; AUC, area under the curve

Table 5 The clinical characteristics of HeFH patients with various genotypes ($n = 105$)

	LDLR null	LDLR defective	APOB/PCSK9	F/H/ χ^2	P value
Patients (n)	49	44	12		
Male, n (%)	25 (51.00%)	24 (54.55%)	6 (50.00%)	0.416	0.961
Age at onset, y	5.67 (2.79, 10.00)	6.66 (4.13, 8.88)	6.71 (3.38, 8.92)	0.258	0.879
Age at diagnosis, y	6.00 (3.21, 10.25)	7.83 (4.55, 9.25)	7.25 (4.09, 9.59)	0.284	0.868
Age at inclusion, y	8.71 (5.84, 13.00)	9.33 (6.58, 10.84)	8.46 (5.09, 11.13)	0.406	0.816
BMI (kg/m ²)	15.77 (14.81, 17.32)	15.00 (14.41, 17.30)	15.72 (14.38, 15.84)	1.598	0.450
BMI SDS	-0.15 \pm 0.28	-0.04 \pm 0.31	-0.35 \pm 0.30	0.161	0.851
Xanthomas, n (%)	1 (2.00%)	5 (11.36%)	0	3.478	0.128
Corneal arcus, n (%)	0	0	0		
Increased cIMT, (%)	4 (8.16%)	0	0	3.663	0.169
First-degree family history of HC, n (%)	41 (83.67%)	40 (90.90%)	10 (83.33%)	1.181	0.596
Second-degree family history of HC, n (%)	27 (55.10%)	22 (50.00%)	4 (33.33%)	1.798	0.387
Family history of premature ASCAD, n (%)	9 (18.37%)	7 (15.91%)	1 (8.3%)	0.787	0.660
TC, mmol/L	8.04 (7.51, 9.44)	7.83 (7.13, 8.36)	8.3 (7.25, 9.28)	5.483	0.064
TG, mmol/L	1.03 (0.74, 1.57)	0.84 (0.68, 1.51)	1.00 (0.46, 4.04)	0.243	0.885
LDL-C, mmol/L	5.81 (5.32, 7.21)	5.24 (4.65, 5.99)	5.80 (5.05, 6.53)	11.246	0.004
HDL-C, mmol/L	1.48 (1.23, 1.65)	1.32 (1.1, 1.78)	1.88 (1.77, 1.96)	4.920	0.085
LDL/HDL	4.14 (3.27, 5.09)	3.96 (3.02, 4.57)	2.88 (2.59, 3.09)	6.355	0.042
APOA, mg/d L	129.12 \pm 7.51	124.44 \pm 10.65	150.24 \pm 5.43	0.901	0.414
APOB, mg/d L	153.70 (132.70, 185.00)	137.70 (124.00, 150.00)	153.70 (132.70, 185.00)	4.672	0.097
APOB/APOA	1.47 (0.99, 1.66)	1.07 (0.78, 1.44)	0.96 (0.90, 0.99)	4.832	0.089
Lp (a), mg/d L	15.9 (11.35, 35.28)	18.7 (11.65, 25.35)	-	0.070	0.792
Low-lipid diet, n (%)	40 (81.63%)	37 (84.09%)	12 (100.00%)	2.544	0.296
Lipid-lowering drugs, n (%)	9 (18.37%)	7 (15.91%)	0	2.277	0.343

BMI body mass index, SDS standard deviation score, cIMT carotid intima-media thickness, n number, HC Hypercholesterolemia, ASCVD arteriosclerotic cardiovascular disease, TC total cholesterol, TG triglyceride, LDL-C low-density lipoprotein cholesterol, HDL-C high-density lipoprotein cholesterol, APOA apolipoprotein A, APOB apolipoprotein B, Lp(a) lipoprotein (a). Normally distributed data are presented as means with standard deviations (means \pm SD) and were analyzed using one-way ANOVA, with the corresponding F and P values. Non-normally distributed data are presented as medians with interquartile ranges [medians, (IQRs)] and were compared using the Kruskal–Wallis test, with the corresponding H and P values. Categorical data are expressed as frequencies (%) and were assessed using the χ^2 test or Fisher's exact test, with the corresponding χ^2 and P values

FH is linked to a lifelong risk of increased cIMT and plaque formation beginning in childhood, leading to the early onset and greater intensity of ASCVD [21–23]. In this study, the prevalence of cIMT thickening was 7.14% ($n=10$), with 4 of these patients developing carotid plaques. Among these patients with plaques, the youngest was only 4.33 years old, with a plaque at the right carotid bifurcation. Consistent with this study, 10% of the 61 boys and 29 girls aged 10–19 years with FH were found to have carotid plaque in a population-based study [20]. Moreover, reversely cascading screening to identify affected parents could be facilitated by diagnosing children with FH within their first ten years of life, allowing for early detection before the occurrence of their first cardiovascular events [11, 24]. Therefore, early diagnosis and intervention of this disease in childhood are strongly warranted.

In this study, the diagnostic process for patients ranged from two months to seven years, with only 40.71% (57/140) being diagnosed with FH before the age of 5.

Unlike in adult patients with FH, classic diagnostic criteria, such as xanthomas, corneal arcus, and premature ASCVD, are rarely seen in pediatric population with FH. Diagnosis in these younger patients mainly depends on elevated LDL-C levels and molecular verification [12]. Consistent with this, the majority of patients (72.14%, 101/140) in the present study visited the outpatient clinic for the first time because of elevated lipid levels discovered incidentally during laboratory investigations. However, further genetic testing was declined by over 20 parents of children whose LDL-C levels fell within the range of 3.6 to 4.9 mmol/L, and 18.09% (19/105) of HeFH patients were identified with LDL-C levels in this range. Moreover, among these patients, only 75.00% ($n=105$) were heterozygotes, whereas 25.00% ($n=35$) were homozygotes (including compound heterozygotes and double heterozygotes). These findings suggest that a significant number of pediatric HeFH patients may be either missed or diagnosed with delay. Owing to the absence of classic phenotypes in pediatric HeFH patients,

Table 6 The clinical characteristics of HoFH patients with different genotypes ($n = 35$)

	Double heterozygotes	LDLR compound heterozygotes	LDLR true homozygotes	F/H/ χ^2	P value
Patients (n)	3	27	5		
Male, n (%)	2 (66.67%)	12 (44.44%)	4 (80.00%)	2.363	0.421
Age at onset, y	8.00 (5.04, 8.67)	2.08 (1.42, 3.67)	3.83 (2.67, 5.00)	4.209	0.122
Age at diagnosis, y	8.08 (5.21, 8.79)	3.42 (2.08, 5.46)	4.83 (4.00, 7.00)	2.017	0.297
Age at inclusion, y	9.75 (8.79, 10.88)	6.50 (4.25, 9.63)	9.00 (5.50, 12.00)	2.429	0.365
BMI (kg/m ²)	18.33 (15.08, 21.58)	14.89 (14.42, 15.40)	16.01 (15.42, 16.60)	1.598	0.231
BMI SDS	0.62 ± 1.14	-0.43 ± 0.29	0.13 ± 0.03	0.965	0.403
Xanthomas, n (%)	0	25 (92.6%)	5 (100%)	11.735	0.002
Corneal arcus, n (%)	0	4 (14.8%)	2 (40%)	2.228	0.258
Increased cIMT, (%)	1 (33.3%)	2 (7.4%)	3 (60%)	7.855	0.018
First-degree family history of HC, n (%)	3 (100%)	26 (96.3%)	5 (100%)	1.563	1.000
Second-degree family history of HC, n (%)	2 (66.7%)	16 (59.3%)	2 (40%)	0.923	0.839
Family history of premature ASCAD, n (%)	1 (33.3%)	6 (22.22%)	0	1.590	0.600
TC, mmol/L	8.11 ± 0.80	17.43 ± 1.12	18.99 ± 1.83	4.575	0.018
TG, mmol/L	1.14 ± 0.11	1.14 ± 0.17	1.49 ± 0.55	0.556	0.581
LDL-C, mmol/L	5.25 ± 0.40	14.43 ± 0.98	17.20 ± 1.45	6.632	0.004
HDL-C, mmol/L	1.42 ± 0.17	1.55 ± 0.16	0.97 ± 0.13	0.983	0.390
LDL/HDL	3.54 (3.51, 3.57)	9.45 (5.27, 15.20)	15.50 (13.68, 22.23)	7.053	0.029
APOA, mg/d L	125.50 ± 14.50	101.80 ± 6.54	101.80 ± 6.53	1.436	0.276
APOB, mg/d L	130.50 ± 16.50	284.24 ± 19.94	330.50 ± 16.50	6.486	0.012
APOB/APOA	1.06 ± 0.25	3.17 ± 0.38	3.53 ± 0.42	3.420	0.070
Lp (a), mg/d L	-	20.4 (19.60, 25.48)	28.3 (17.90, 38.7)	1.914	0.203
Low-lipid diet, n (%)	2 (33.3%)	7 (25.9%)	0	3.741	0.137
Lipid-lowering drugs, n (%)	1 (33.3%)	18 (66.7%)	5 (100%)	3.726	0.137
Liver translation, n (%)	0	3 (100%)	0	0.579	1.000

BMI body mass index, SDS standard deviation score, cIMT carotid intima-media thickness, n number, HC Hypercholesterolemia, ASCVD arteriosclerotic cardiovascular disease, TC total cholesterol, TG triglyceride, LDL-C low-density lipoprotein cholesterol, HDL-C high-density lipoprotein cholesterol, APOA apolipoprotein A, APOB apolipoprotein B, Lp(a) lipoprotein (a). Normally distributed data are presented as means with standard deviations (means ± SD) and were analyzed using one-way ANOVA, with the corresponding F and P values. Non-normally distributed data are presented as medians with interquartile ranges [medians, (IQRs)] and were compared using the Kruskal–Wallis test, with the corresponding H and P values. Categorical data are expressed as frequencies (%) and were assessed using the χ^2 test or Fisher's exact test, with the corresponding χ^2 and P values

most cases remain undiagnosed until midlife or even following the first cardiovascular event, which delays timely intervention [25]. Without timely treatment, lipid levels remain persistently elevated, leading to an ASCVD incidence that is three to four times higher in FH individuals relative to non-FH individuals, with cardiovascular disease (CVD) developing approximately 10 years earlier [26]. Additionally, although the presence of xanthomas served as the initial diagnostic indicator for the majority of HoFH patients (74.29%, 26/35), their average diagnostic timeline was 18.84 months. Furthermore, 5 of the patients were not identified until after undergoing skin biopsies, indicating under-recognition of FH phenotypic indicators by medical professionals and parents. Therefore, there are three recommendations to facilitate the early detection and therapeutic intervention for FH: first, increase awareness of the disease among doctors and parents; second, as advised by the European Atherosclerosis

Society (EAS), children between 2 and 10 years should be screened using fasting LDL-C levels, adhering to the clinical diagnostic guidelines for pediatric FH, which include LDL-C concentrations ≥ 3.6 mmol/L, along with signs such as xanthomas, corneal arcus, premature ASCVD, or a familial history of hypercholesterolemia and/or early-onset ASCVD in immediate and extended relatives; and third, conduct genetic screening for individuals meeting the clinical diagnostic criteria [7, 13, 27].

Through genetic testing, 87 different variants were identified among 140 cases, with LDLR variants accounting for 91.95%, APOB variants accounting for 6.90%, and PCSK9 variants accounting for 1.15%, which was consistent with other studies [28]. Notably, 11 variants (12.64%), including 6 frameshift variants, 2 missense variants, 2 exon deletions, and 1 nonsense variant, found in this study have never been reported, suggesting that the FH-causing mutation spectrum continues to expand.

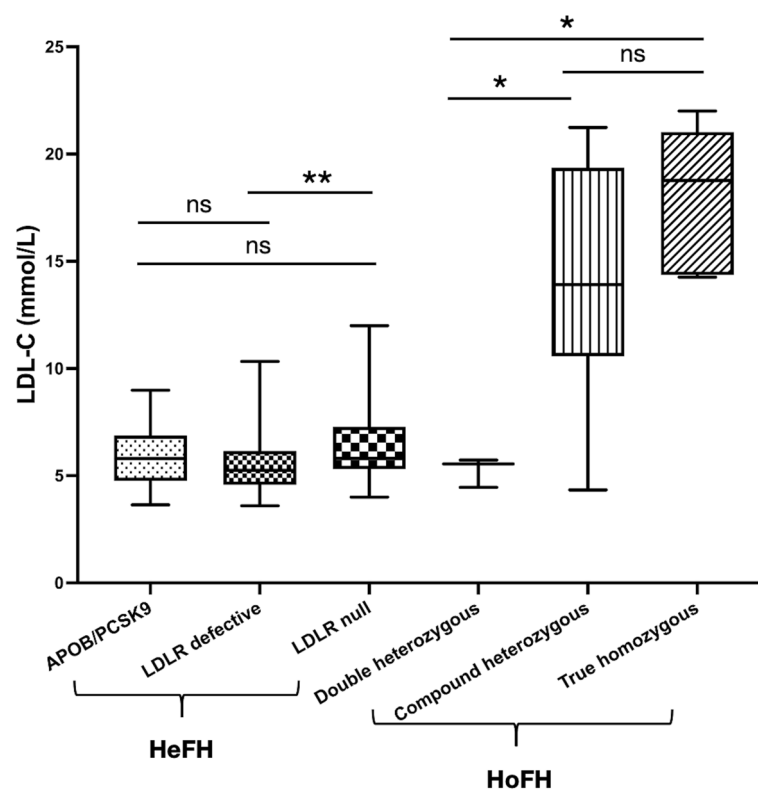


Fig. 4 Low-density lipoprotein-cholesterol levels in patients with different HeFH and HoFH types. ns, not significant; HeFH, heterozygous familial hypercholesterolemia; HoFH, homozygous familial hypercholesterolemia

Furthermore, frameshift variants caused by the insertion or deletion of one or more bases were the most common novel variants in the present study, unlike previous studies in which missense variants were the most common among novel variants [29, 30]. Since frameshift variants have greater pathogenicity and are more likely to be detected than missense variants, a subset of patients with a mild phenotype, such as those whose LDL-C levels fell within the range of 3.6 to 4.9 mmol/L, may not have been included in the study.

According to the ACMG and ClinGen guidelines, 13 VUS (11 missense variants, 1 splicing variant and 1 in-frame deletion/insertion) and 1 likely benign missense variant were identified in the study. To assess their pathogenicity, in silico analysis tools, such as PolyPhen-2, SIFT, and mutation testers, have been utilized to predict changes in protein structure and function. Additionally, the phenotypes and family histories of patients with VUS or LB variants were analyzed. All patients with these 14 variants exhibited LDL-C concentrations ≥ 3.6 mmol/L, and their immediate or extended family members with the same variants also exhibited hypercholesterolemia. Furthermore, the lipid levels of these patients were similar to those found in individuals with pathogenic or

likely pathogenic variants. However, ex vivo functional analysis and in silico predictions suggested that some of these VUSs and likely benign variants have no adverse effects on LDLR function [31, 32]. Therefore, there is a need for additional insights from laboratory-based functional assays and clinical studies of FH patients with VUS and LB variants to determine their pathogenicity and classification.

Furthermore, 91.25% ($n=73$) of the variants of LDLR were located in exons that encode ligand binding domains and EGF precursor homology domains. In particular, the most common variants was in exon 4, which encodes the APOB binding domain, consistent with findings from other studies [27]. In addition, this study demonstrated that the 4 most prevalent LDLR variants were c.1448G>A (13.57%), c.1747C>T (10.00%), c.1879G>A (10.00%) and c.1216C>A (9.29%), accounting for 42.86% of the patients. W483* and H583Y were exclusively observed in Asian populations, and A627T and R406R were not found outside of Chinese individuals [28, 29]. This distribution may be attributed to a founder effect, aligning with previous studies describing FH-related variants in China [33–36]. Moreover, a hotspot variant of the APOB gene, c.10579C>T, which accounts for

Table 7 The characteristics of HeFH patients with common variants and other variants (*n* = 105)

	LDLR			APOB	Other variants	F/H/ χ^2	P value
	c.1747C>T	c.1448G>A	c.1216C>A	c.10579C>T			
Patients (n)	10	12	12	8	63		
Male, n (%)	5 (50.0%)	6 (50%)	8 (66.7%)	5 (62.5%)	31 (49.2%)	1.682	0.837
Age at onset, y	7.52 ± 0.94	4.55 ± 1.21	7.13 ± 1.13	4.77 ± 1.07	6.70 ± 0.48	1.532	0.199
Age at diagnosis, y	7.81 ± 0.92	4.80 ± 1.22	7.43 ± 1.17	5.50 ± 1.18	7.33 ± 0.47	1.57	0.188
Age at inclusion, y	9.05 ± 1.02	7.11 ± 1.33	8.99 ± 1.30	7.02 ± 1.28	9.65 ± 0.60	1.207	0.313
BMI (kg/m ²)	19.67 ± 3.40	16.46 ± 1.11	16.01 ± 0.85	14.88 ± 0.51	15.84 ± 0.43	1.959	0.117
Xanthomas, n (%)	1 (10.0%)	0	0	0	5 (7.9%)	1.754	0.753
Corneal arcus, n (%)	0	0	0	0	0		
Increased cIMT, (n (%))	0	0	1 (8.3%)	0	3 (4.8%)	1.645	0.875
First-degree family history of HC, n (%)	8 (80.0%)	10 (83.3%)	10 (83.3%)	7 (87.5%)	56 (88.9%)	1.762	0.846
Second-degree family history of HC, n (%)	3 (30.0%)	8 (66.7%)	7 (58.3%)	3 (37.5%)	32 (50.8%)	3.692	0.465
First-degree family history of ASCVD, n (%)	0	2 (16.7%)	2 (16.7%)	0	2 (3.2%)	5.977	0.107
Second-degree family history of ASCVD, n (%)	1 (10.0%)	1 (8.3%)	3 (25%)	1 (12.5%)	5 (7.9%)	3.52	0.393
TC, mmol/L	7.20 (6.15, 7.97)	7.77 (7.52, 8.19)	8.83 (7.57, 10.50)	9.08 (7.01, 11.00)	8.00 (7.30, 8.60)	6.882	0.142
TG, mmol/L	0.81 (0.72, 1.06)	0.99 (0.69, 1.15)	1.03 (0.72, 1.25)	0.70 (0.54, 2.83)	1.01 (0.72, 1.51)	0.696	0.952
LDL-C, mmol/L	4.91 (4.42, 5.25)	5.76 (5.59, 6.30)	5.83 (4.91, 7.44)	6.19 (5.51, 7.47)	5.65 (5.07, 6.37)	7.12	0.13
HDL-C, mmol/L	1.74 (1.10, 1.78)	1.44 (1.19, 1.65)	1.60 (1.34, 2.05)	-	1.43 (1.21, 1.63)	4.146	0.387
LDL/HDL	3.02 (2.95, 3.44)	4.58 (3.18, 4.90)	3.86 (3.44, 4.72)	-	3.98 (3.27, 4.82)	4.145	0.387
APOA, mg/dl	141.54 ± 18.92	147.55 ± 20.83	141.78 ± 9.03	-	118.10 ± 8.09	1.051	0.395
APOB, mg/dl	130 (128.00, 137.70)	138.60 (124.60, 150.00)	182.85 (138.40, 222.70)	-	148 (126.50, 171.40)	4.537	0.338
APOB/APOA	0.94 (0.82, 1.07)	0.81 (0.78, 1.26)	1.32 (0.90, 1.74)	-	1.42 (0.97, 1.55)	3.688	0.45
Lp (a), mg/dl	15.50 (14.00, 26.20)	27.55 (11.50, 40.42)	17.05 (12.20, 21.65)	-	16.85 (11.03, 23.28)	3.18	0.528
low-lipid diet, n (%)	10 (100.0%)	12 (100.0%)	9 (75.0%)	8 (100.0%)	50 (79.4%)	6.424	0.114
Lipid-lowering drugs, n (%)	0	0	3 (25%)	0	13 (20.6%)	6.424	0.114

BMI body mass index, SDS standard deviation score, cIMT carotid intima-media thickness, n number, HC Hypercholesterolemia, ASCVD arteriosclerotic cardiovascular disease, TC total cholesterol, TG triglyceride, LDL-C low-density lipoprotein cholesterol, HDL-C high-density lipoprotein cholesterol, APOA apolipoprotein A, APOB apolipoprotein B, Lp(a) lipoprotein (a). Normally distributed data are presented as means with standard deviations (means ± SD) and were analyzed using one-way ANOVA, with the corresponding F and P values. Non-normally distributed data are presented as medians with interquartile ranges [medians, (IQRs)] and were compared using the Kruskal–Wallis test, with the corresponding H and P values. Categorical data are expressed as frequencies (%) and were assessed using the χ^2 test or Fisher's exact test, with the corresponding χ^2 and P values

6.43% (9/140) of all the identified variants, has also been reported in other studies from China [29, 33–35]. In contrast, the c.10708C>T (p.R3527Q) variant is the most prevalent APOB variant in Caucasian populations [37]. This variant is located in exon 26, encoding an important domain that functions as a ligand for LDLR during receptor-mediated endocytosis. It is associated with relatively high LDL-C and TC concentrations, together with the broadest range of LDL-C values observed in the study. All 5 variants are predominantly found in Asia, especially in China, suggesting their potential importance in Chinese newborn genetic screening for FH [38].

When phenotype severity was correlated with genotype, patients with HoFH clearly presented 2–threefold higher lipid levels [TC, LDL-C, APOB, APOB/APOA, and Lp(a)] than did with HeFH did, in agreement with the current investigation [39]. The LDL-C/HDL-C ratio, which underscores the primary roles of both LDL-C and HDL-C in FH patients, is also greater in HoFH patients than in HeFH patients, indicating greater potential for coronary heart disease [40]. The APOA, which is the primary protein component of HDL particles and a key regulator in lipid metabolism, is much lower in HeFH patients than in HoFH patients [41]. Furthermore,

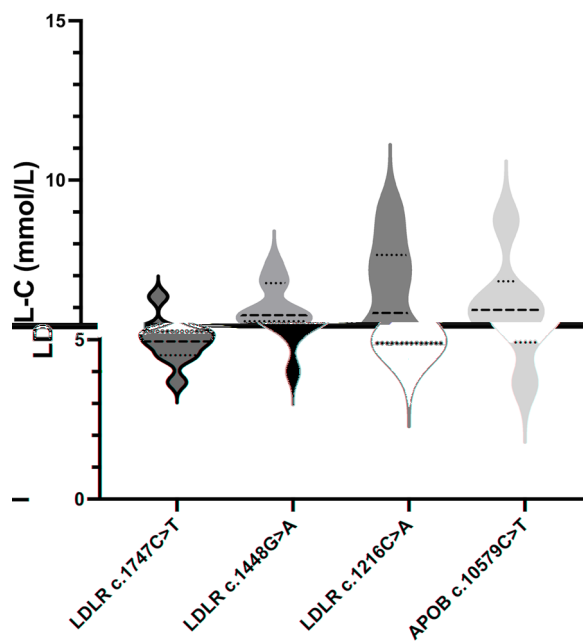


Fig. 5 Violin plots reporting the low-density lipoprotein cholesterol (LDL-C) levels observed among patients with the 4 most frequent pathogenic variants. The violin shape represents the smoothed frequency distribution of the LDL-C values expressed in mmol/L. The continuous horizontal line within each value represents the distribution median, whereas the dashed lines represent the first and third quartiles of the value distribution in each group

40.00% (14/35) of HoFH patients exhibited LDL-C levels ≤ 13 mmol/L, despite the EAS consensus statement suggesting diagnostic criteria of LDL-C > 13 mmol/L accompanied by evidence of xanthomas in early childhood (before age 10) [6, 42]. Consequently, an optimal LDL-C cutoff value of 10.34 mmol/L was determined through ROC curve analysis to accurately distinguish HoFH patients from HeFH patients, which corresponds to the LDL-C level of 10 mmol/L observed in other studies [6, 28]. In addition, individuals with multiple FH-causing variants were diagnosed at an earlier age and presented a greater prevalence of xanthomas, corneal arcus, and increased carotid intima-media thickness. Moreover, 75.00% of the patients (3/4) with plaques were HoFH patients, and the youngest patient who developed a plaque at 4.33 years of age was a compound heterozygote for FH. Overall, the genotype of HoFH is associated with a more severe phenotype and a greater likelihood of ASCVD, emphasizing the significance of early diagnosis and intervention [43].

In this study, among the HoFH patients, the mean levels of the main lipid profiles increased in the following order: true LDLR homozygotes $>$ compound LDLR heterozygotes $>$ double heterozygotes, which is consistent

with previous research [44]. Interestingly, compared with true homozygotes and compound LDLR heterozygotes, double heterozygotes presented a milder phenotype resembling that of monogenic FH, with a significantly lower lipid profile and a lower incidence of xanthomas. This finding contrasts with previous studies, which could be attributed to a combination of the limited patient cohort and variant heterogeneity [1]. For the different variants, the LDL-C levels, APOB levels, and LDL-C/HDL-C ratios in HeFH patients with null variants were greater than those in HeFH patients with APOB, PCSK9, or defective LDLR variants, which concurs with prior findings and suggests that more severe LDLR dysfunction is associated with these null variants [45]. Based on the observed genotype-phenotype correlations, the severity of the FH phenotype can be predicted through genetic testing and implementing more aggressive interventions for true LDLR homozygotes, compound LDLR heterozygotes and heterozygotes with null variants owing to their higher lipid levels and increased risk of plaque formation.

Given the significantly elevated risk of early-onset ASCVD, the necessity and critical importance of early intervention cannot be overstated. According to clinical guidelines for managing hypercholesterolemia in pediatric populations, lifestyle and dietary interventions are the first-line treatments for clinically or genetically diagnosed FH patients [9]. For HeFH children aged ≥ 8 years, medication therapy, typically using statins, is recommended if LDL-C concentrations remain ≥ 4.91 mmol/L after 6 months of lifestyle and dietary modifications [46]. Moreover, HoFH children could start lipid-lowering drugs as early as two years of age and consider lipoprotein apheresis or liver transplantation if pharmacotherapy is not effective [42]. In the present study, FH patients were undertreated, with only 15.24% (16/105) of HeFH patients and 68.57% (24/35) of HoFH patients receiving pharmacologic treatment. Additionally, only 3 compound heterozygous patients underwent liver transplantation. More concerning, among the HoFH patients receiving lipid-lowering therapy, 33.33% ($n=8$) initiated treatment after the age of 8, suggesting a significant prevalence of treatment delays. In one respect, since statins, the first-line lipid-lowering drugs, lack pediatric indications in China and ezetimibe is not approved for children under 10 years of age for clinical use, physicians are not sufficiently familiar with the treatment strategies for these medications and are often hesitant to prescribe them. Conversely, parental adherence to LLT in children with FH was suboptimal owing to concerns about potential adverse effects of the medications, the risk of malnutrition from a cholesterol-restricted diet and insufficient

awareness of the risks associated with hypercholesterolemia and premature ASCVD. Therefore, additional studies with large cohorts are required to validate the safety and effectiveness of lipid-lowering therapies and to improve early intervention strategies.

Strengths and limitations

This study’s strengths are highlighted by the inclusion of a large, diverse sample of pediatric patients with familial hypercholesterolemia from multiple centers across China. Furthermore, all patients underwent comprehensive lipid profile testing and genetic screening, and follow-up data on lipid-lowering treatment regimens were collected. Although this study offers valuable insights into the genotype and genotype–phenotype relationships within the Chinese pediatric population, it is subject to several limitations that must be recognized. First, this study comprises the largest cohort from multiple centers across mainland China to date, the number of patients remains small, particularly for those with HeFH patients. Owing to the milder phenotype in HeFH patients and the reluctance of some parents to pursue further genetic testing, a significant number of cases may have been misdiagnosed or diagnosed with delays. This introduces potential bias in the participant pool and underscores the need for a larger sample size in future study to enhance the representativeness and reliability of the findings. Second, some values for lipid profiles and family history of hypercholesterolemia were recorded on the basis of parents’ recall, which may not have been accurate. Third, functional analyses of LDL receptors were not performed on the novel variants identified in this study. Finally, 4 clinically diagnosed probands without detectable variants were identified through whole-exome sequencing. This may suggest the presence of undiscovered disease-causing genes or a polygenic basis for hypercholesterolemia in these patients. Additionally, environmental and lifestyle factors also contribute to clinically defined FH. Therefore, further work should target these patients to identify potentially new causative genes and polygenic susceptibility to elevated plasma LDL-C [47].

Conclusion

In summary, the genotypes and phenotypes of FH in the Chinese pediatric population exhibit significant variability. Additionally, the genetic profile of the Chinese population differs from that of Caucasians, offering a valuable dataset for the clinical genetic screening of FH in China. Furthermore, the findings emphasize the need for earlier diagnosis and treatment of FH in Chinese pediatric patients, as indicated by the older age at diagnosis and treatment in this cohort.

To address this, improved early identification through lipid or genetic screening, along with the implementation of timely and regular pharmacological treatments, is crucial to better manage and reduce the burden of FH in this population.

Abbreviations

ABCG5	ATP-binding cassette subfamily G member 5
ABCG8	ATP-binding cassette subfamily G member 8
APOA	Apolipoprotein A
APOB	Apolipoprotein B
APOE	Apolipoprotein E
ASCVD	Arteriosclerotic cardiovascular disease
BMI	Body mass index
ciMT	Carotid intima–media thickness
EAS	European Atherosclerosis Society
FH	Familial hypercholesterolemia
HDL-C	High-density lipoprotein cholesterol
HeFH	Heterozygous familial hypercholesterolemia
HoFH	Homozygous familial hypercholesterolemia
LB	Likely benign
LDL-C	Low-density lipoprotein cholesterol
LDLR	Low-density lipoprotein receptor
LDLRAP	LDLR adaptor protein 1
LLT	Lipid-lowering therapy
LP	Likely pathogenic
Lp(a)	Lipoprotein (a)
NGS	Next-generation sequencing
P	Pathogenic
PCSK9	Proprotein convertase subtilisin/Kexin type 9
SDS	Standard deviation score
STAP1	Signal-transducing adaptor family member 1
TC	Total cholesterol
TG	Triglyceride
VUS	Variants of uncertain significance
WES	Whole exome sequencing

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12944-024-02406-4>.

Supplementary Material 1.
Supplementary Material 2.
Supplementary Material 3.
Supplementary Material 4.

Acknowledgements

We are grateful to the patient and their parents for granting us permission to use the data.

Authors’ contributions

Zou CC and Fu JF supervised the study. Wang CC, Ma MS, Chi MZ, Zhou Q, Jiang LH, Wang CL, Lu M, Chen XQ, Cheng YY, Ke Q, Wang DY, Qian XX, Ying XM, Zhang JP, Shen QH, Liu LF, Gu R, Zhang ZJ, Feng JH, Wang M, Zhu MQ and Huang K contributed to the data collection and patient management. Huang MN wrote the original draft. All the authors read and approved the final manuscript.

Funding

This work is supported by the National Natural Science Foundation of China (81670786) and the Zhejiang Province Medical and Health Leading Talent Project (2024).

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

The study received approval from the Ethics Committee of the Children's Hospital of Zhejiang University School of Medicine. Written informed consent was obtained from all participants or their legal guardians prior to the performance of whole exome sequencing (WES).

Competing interests

The authors declare no competing interests.

Author details

¹Department of Endocrinology, Children's Hospital of Zhejiang University School of Medicine, National Clinical Research Center for Child Health, Hangzhou, Zhejiang 310052, China. ²Department of Pediatrics, The First People's Hospital of Yongkang Affiliated to Hangzhou Medical College, Jinhua, China. ³Department of Pediatrics, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing, China. ⁴Department of Pediatrics, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, China. ⁵Department of Endocrinology, Genetics and Metabolism, Fujian Children's Hospital, Fuzhou, China. ⁶Department of Pediatrics, General Hospital of Tianjin Medical University, Tianjin, China. ⁷Department of Pediatrics, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China. ⁸Department of Pediatrics, Women and Children's Hospital, School of Medicine, Xiamen University, Xiamen, China. ⁹Department of Pediatrics, The Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China. ¹⁰Department of Pediatrics, Hebei General Hospital, Shijiazhuang, China. ¹¹Department of Neurology, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China. ¹²Department of Pediatrics, Jinhua Maternal and Child Health Hospital, Jinhua, China. ¹³Department of Pediatrics, Shengzhou People's Hospital, Shengzhou, China. ¹⁴Department of Pediatrics, The First People's Hospital of Taizhou, Taizhou, China. ¹⁵Department of Pediatrics, Ningbo Women and Children's Hospital, Ningbo, China. ¹⁶Department of Pediatrics, Haining People's Hospital, Haining, China. ¹⁷Department of Pediatrics, Lishui Maternal and Child Health Care Hospital, Lishui, China. ¹⁸Department of NICU, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, China.

Received: 26 August 2024 Accepted: 13 December 2024

Published online: 27 December 2024

References

- Sun D, Zhou BY, Li S, et al. Genetic basis of index patients with familial hypercholesterolemia in Chinese population: mutation spectrum and genotype-phenotype correlation. *Lipids Health Dis*. 2018;17(1):252.
- Defesche JC, Gidding SS, Harada-Shiba M, et al. Familial hypercholesterolemia. *Nat Rev Dis Primers*. 2017;3:17093.
- Abifadel M, Boileau C. Genetic and molecular architecture of familial hypercholesterolemia. *J Intern Med*. 2023;293(2):144–65.
- Fourgeaud M, Lebreton L, Belabbas K, et al. Phenotypic and genotypic characterization of familial hypercholesterolemia in French adult and pediatric populations. *J Clin Lipidol*. 2022;16(3):298–305.
- Santos RD, Gidding SS, Hegele RA, et al. Defining severe familial hypercholesterolemia and the implications for clinical management: a consensus statement from the International Atherosclerosis Society Severe Familial Hypercholesterolemia Panel. *Lancet Diabetes Endocrinol*. 2016;4(10):850–61.
- Cuchel M, Raal FJ, Hegele RA, et al. 2023 Update on European Atherosclerosis Society Consensus Statement on Homozygous Familial Hypercholesterolemia: new treatments and clinical guidance. *Eur Heart J*. 2023;44(25):2277–91.
- Nordestgaard BG, Chapman MJ, Humphries SE, et al. Familial hypercholesterolemia is underdiagnosed and undertreated in the general population: guidance for clinicians to prevent coronary heart disease: consensus statement of the European Atherosclerosis Society. *Eur Heart J*. 2013;34(45):3478–90.
- Hu P, Dharmayat KI, Stevens CAT, et al. Prevalence of Familial Hypercholesterolemia Among the General Population and Patients with Atherosclerotic Cardiovascular Disease: A Systematic Review and Meta-Analysis. *Circulation*. 2020;141(22):1742–59.
- Cuchel M, Bruckert E, Ginsberg HN, et al. Homozygous familial hypercholesterolemia: new insights and guidance for clinicians to improve detection and clinical management. A position paper from the Consensus Panel on Familial Hypercholesterolemia of the European Atherosclerosis Society. *Eur Heart J*. 2014;35(32):2146–57.
- Sustar U, Kordonouri O, Mlinaric M, et al. Universal screening for familial hypercholesterolemia in 2 populations. *Genet Med*. 2022;24(10):2103–11.
- EAS Familial Hypercholesterolemia Studies Collaboration, Vallejo-Vaz AJ, De Marco M, et al. Overview of the current status of familial hypercholesterolemia care in over 60 countries - The EAS Familial Hypercholesterolemia Studies Collaboration (FHSC). *Atherosclerosis*. 2018;277:234–55.
- European Atherosclerosis Society Familial Hypercholesterolemia Studies Collaboration. Familial hypercholesterolemia in children and adolescents from 48 countries: a cross-sectional study. *Lancet*. 2024;403(10421):55–66.
- Atherosclerosis and Coronary Heart Disease Group of the Chinese Society of Cardiology of Chinese Medical Association; Editorial Board of Chinese Journal of Cardiology. *Zhonghua Xin Xue Guan Bing Za Zhi*. 2018;46(2):99–103.
- Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17(5):405–24.
- Chora JR, Iacocca MA, Tichý L, et al. The Clinical Genome Resource (ClinGen) Familial Hypercholesterolemia Variant Curation Expert Panel consensus guidelines for LDLR variant classification. *Genet Med*. 2022;24(2):293–306.
- Sánchez-Hernández RM, Civeira F, Stef M, et al. Homozygous Familial Hypercholesterolemia in Spain: Prevalence and Phenotype-Genotype Relationship. *Circ Cardiovasc Genet*. 2016;9(6):504–10.
- Gu R, Wang H, Wang CL, et al. Gene variants and clinical characteristics of children with sitosterolemia. *Lipids Health Dis*. 2024;23(1):83.
- Tonstad S, Joakimsen O, Stensland-Bugge E, et al. Risk factors related to carotid intima-media thickness and plaque in children with familial hypercholesterolemia and control subjects. *Arterioscler Thromb Vasc Biol*. 1996;16(8):984–91.
- Jiang L, Benito-Vicente A, Tang L, et al. Analysis of LDLR variants from homozygous FH patients carrying multiple mutations in the LDLR gene. *Atherosclerosis*. 2017;263:163–70.
- Etchebarria A, Benito-Vicente A, Stef M, et al. Activity-associated effect of LDL receptor missense variants located in the cysteine-rich repeats. *Atherosclerosis*. 2015;238(2):304–12.
- Minicocci I, Pozzessere S, Prisco C, et al. Analysis of Children and Adolescents with Familial Hypercholesterolemia. *J Pediatr*. 2017;183:100–107.e3.
- Santos RD, Miname MH. Increased subclinical atherosclerosis burden in familial hypercholesterolemia phenotype: What do genetic defects tell us and what are the clinical implications? *Atherosclerosis*. 2017;263:316–7.
- Benn M, Watts GF, Tybjaerg-Hansen A, Nordestgaard BG. Familial hypercholesterolemia in the Danish general population: prevalence, coronary artery disease, and cholesterol-lowering medication. *J Clin Endocrinol Metab*. 2012;97(11):3956–64.
- Berberich AJ, Hegele RA. The complex molecular genetics of familial hypercholesterolemia. *Nat Rev Cardiol*. 2019;16(1):9–20.
- Chen P, Chen X, Zhang S. Current status of familial hypercholesterolemia in China: a need for patient FH registry systems. *Atherosclerosis*. 2019;10:280.
- Huijgen R, Kindt I, Defesche JC, et al. Cardiovascular risk in relation to functionality of sequence variants in the gene coding for the low-density lipoprotein receptor: a study among 29,365 individuals tested for 64 specific low-density lipoprotein-receptor sequence variants. *Atherosclerosis*. 2012;233(2):2325–30.
- Harada-Shiba M, Ohta T, Ohtake A, et al. Guidance for Pediatric Familial Hypercholesterolemia 2017. *J Atheroscler Thromb*. 2018;25(6):539–53.
- Jiang L, Stoekenbroek RM, Zhang F, et al. Homozygous familial hypercholesterolemia in China: Genetic and clinical characteristics from a real-world, multi-center, cohort study. *J Clin Lipidol*. 2022;16(3):306–14.
- Jiang L, Sun LY, Dai YF, Yang SW, Zhang F, Wang LY. The distribution and characteristics of LDL receptor mutations in China: A systematic review. *Sci Rep*. 2015;5:17272.

30. Leigh S, Futema M, Whittall R, et al. The UCL low-density lipoprotein receptor gene variant database: pathogenicity update. *J Med Genet*. 2017;54(4):217–23.
31. Graça R, Alves AC, Zimon M, et al. Functional profiling of LDLR variants: Important evidence for variant classification: Functional profiling of LDLR variants. *J Clin Lipidol*. 2022;16(4):516–24.
32. Alharbi KK, Kashour TS, Al-Hussaini W, et al. Screening for genetic mutations in LDLR gene with familial hypercholesterolemia patients in the Saudi population. *Acta Biochim Pol*. 2015;62(3):559–62.
33. Xiang R, Fan LL, Lin MJ, et al. The genetic spectrum of familial hypercholesterolemia in the central south region of China. *Atherosclerosis*. 2017;258:84–8.
34. Jiang L, Sun LY, Pan XD, et al. Characterization of the unique Chinese W483X mutation in the low-density lipoprotein-receptor gene in young patients with homozygous familial hypercholesterolemia. *J Clin Lipidol*. 2016;10(3):538–546.e5.
35. Jingxin S, Shitong C. Expanding the genetic spectrum for Chinese familial hypercholesterolemia population with six genetic mutations identified using a next-generation sequencing-based laboratory-developed screening test. *Mol Genet Genomic Med*. 2022;10(12):e2070.
36. Defesche JC, Schuurman EJ, Klaaijsen LN, et al. Silent exonic mutations in the low-density lipoprotein receptor gene that cause familial hypercholesterolemia by affecting mRNA splicing. *Clin Genet*. 2008;73(6):573–8.
37. Rutkowska L, Salacińska K, Salachna D, et al. Identification of New Genetic Determinants in Pediatric Patients with Familial Hypercholesterolemia Using a Custom NGS Panel. *Genes (Basel)*. 2022;13(6):999.
38. Kalra S, Chen Z, Deerochanawong C, et al. Familial Hypercholesterolemia in Asia Pacific: A Review of Epidemiology, Diagnosis, and Management in the Region. *J Atheroscler Thromb*. 2021;28(5):417–34.
39. Cameron J, Holla OL, Laerdahl JK, et al. Characterization of novel mutations in the catalytic domain of the PCSK9 gene. *J Intern Med*. 2008;263(4):420–31.
40. Björnsson E, Thorgeirsson G, Helgadóttir A, et al. Large-Scale Screening for Monogenic and Clinically Defined Familial Hypercholesterolemia in Iceland. *Arterioscler Thromb Vasc Biol*. 2021;41(10):2616–28.
41. Carmena-Ramon RF, Ordoñas JM, Ascaso JF, et al. Influence of genetic variation at the apo A-I gene locus on lipid levels and response to diet in familial hypercholesterolemia. *Atherosclerosis*. 1998;139(1):107–13.
42. Tromp TR, Hartgers ML, Hovingh GK, et al. Worldwide experience of homozygous familial hypercholesterolemia: retrospective cohort study. *Lancet*. 2022;399(10326):719–28.
43. Di Taranto MD, de Falco R, Guardamagna O, et al. Lipid profile and genetic status in a familial hypercholesterolemia pediatric population: exploring the LDL/HDL ratio. *Clin Chem Lab Med*. 2019;57(7):1102–10.
44. Sanna C, Stéphenne X, Revencu N, et al. Homozygous familial hypercholesterolemia in childhood: Genotype-phenotype description, established therapies and perspectives. *Atherosclerosis*. 2016;247:97–104.
45. Junyent M, Gilabert R, Jaraúta E, et al. Impact of low-density lipoprotein receptor mutational class on carotid atherosclerosis in patients with familial hypercholesterolemia. *Atherosclerosis*. 2010;208(2):437–41.
46. Subspecialty Group of Rare Diseases, the Society of Pediatrics, Chinese Medical Association; Subspecialty Group of Cardiology, the Society of Pediatrics, Chinese Medical Association; Subspecialty Group of Child Health Care, the Society of Pediatrics, Chinese Medical Association; Subspecialty Group of Endocrinological, Hereditary and Metabolic Diseases, the Society of Pediatrics, Chinese Medical Association; Editorial Board, Chinese Journal of Pediatrics. *Zhonghua Er Ke Za Zhi*. 2022;60(7):633–9.
47. Ramaswami U, Humphries SE, Priestley-Barnham L, et al. Current management of children and young people with heterozygous familial hypercholesterolemia - HEART UK statement of care. *Atherosclerosis*. 2019;290:1–8.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.