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The distribution and characteristics of LDL receptor mutations in China: A systematic review

Long Jiang¹, Li-Yuan Sun^{1,2}, Yan-Fang Dai¹, Shi-Wei Yang¹, Feng Zhang^{3,4} & Lu-Ya Wang¹

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Familial hypercholesterolemia (FH) is a common and serious dominant genetic disease, and its main pathogenic gene is the low-density lipoprotein receptor (LDLR) gene. This study aimed to perform a systematic review of LDLR mutations in China. Using PubMed, Embase, Wanfang (Chinese), the Chinese National Knowledge Infrastructure (Chinese), and the Chinese Biological and Medical database (Chinese), public data were limited to December 2014. The Medical Subject Headings terms and the following key words were used: "familial hypercholesterolemia", "Chinese", "China", "Hong Kong", and "Taiwan". A total of 74 studies including 295 probands with 131 LDLR mutations were identified. Most of the mutations were located in exon 4 of LDLR and approximately 60% of the mutations were missense mutations. Thirty new mutations that were not recorded in the LDLR databases were found. In silico analysis revealed that most of the mutations were pathogenic. The primary LDLR mutations were C308Y, H562Y, and A606T, and all of the mutations had functional significance. Prevalence data suggest that there are nearly 3.8 million FH patients in China, although reported numbers are much smaller, suggesting that FH is widely misunderstood. This systematic review provides information that is specific to China for inclusion in the international FH database.

Familial hypercholesterolemia (FH, OMIM: #143890), which is characterized by tendon xanthoma, severely elevated LDL cholesterol (LDL-C) and premature coronary heart disease (pCHD), is a common and serious dominant genetic disease and has recently become a topic of extensive concern worldwide¹. LDL-C levels are elevated 2- to 3-fold in heterozygous FH (HeFH) patients, and these patients progress to CHD before age 45 if they are not treated. Homozygous FH (HoFH) patients exhibit 6- to 8-fold increases in plasma LDL-C and a severe phenotype; these patients also develop serious cardiovascular disease before the age of 12.5 years if untreated². Recently, HeFH and HoFH were found to occur in approximately 1/200 to 1/500 of the population and 1/160 000 to 1/300 000 of the population, respectively, which is higher than previously reported values³. One Chinese study reported that the prevalence of probable/definite FH was 0.28% (1.4/500) based on the modified Dutch lipid clinic network (DLCN) definition, which is similar to the worldwide prevalence⁴. Hence, there are nearly 36 million potential FH patients in the world, including 3.8 million patients in China. However, current data have shown that FH is underdiagnosed and undertreated in most counties, especially in mainland China, where only ~100 index patients have been reported⁵. Therefore, there is likely a lack of understanding of FH among the general public in mainland China.

¹Beijing Anzhen Hospital, Affiliated to Capital Medical University, Beijing Institute of Heart, Lung and Blood Vessel Diseases. The Key Laboratory of Remodelling-related Cardiovascular Diseases, Ministry of Education, Department of Atherosclerosis, Beijing 100029, China. ²Beijing Anzhen Hospital, Affiliated to Capital Medical University, Department of Dermatology, Beijing 100029, China. ³Institute of Genomics, Chinese Academy of Sciences and Key Laboratory of Genome Science and Information, Chinese Academy of Sciences, Beijing 100101, China. ⁴National Engineering Laboratory for Druggable Gene and Protein Screening, Northeast Normal University, Changchun 130024, Jilin, China. Correspondence and requests for materials should be addressed to L.Y.W. (email: azywly@163.com)

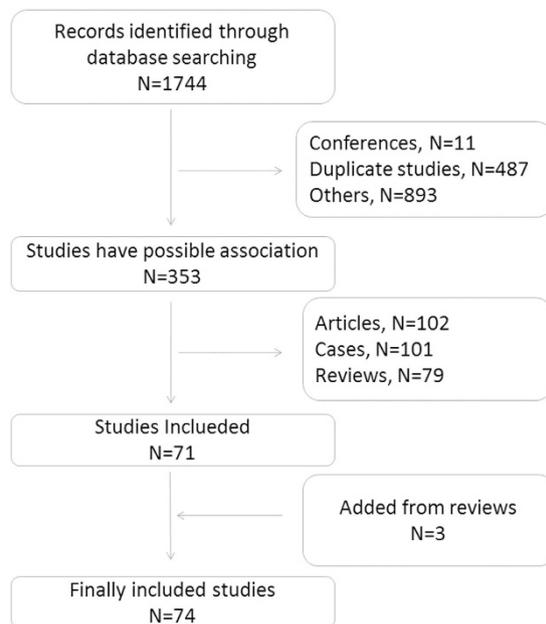


Figure 1. Flow chart of the study selection.

FH is a monogenic autosomal dominant disease in which a single causative mutation in the pathogenic gene affects cholesterol metabolism. The major pathogenic genes for FH are low-density lipoprotein receptor (LDLR, MIM 606945), apolipoprotein B (APOB, MIM107730), and proprotein convertase subtilisin/kexin type 9 (PCSK9, MIM 607786)¹. The most important pathogenic gene is LDLR, and approximately 90% of FH patients have mutations in this gene. The other pathogenic genes have been reported less frequently worldwide, especially in China. Based on the current LOVD databases (www.ucl.ac.uk/ldlr/LOVDv.1.1.0/ and https://grenada.lumc.nl/LOVD2/UCLHeart/home.php?select_db=LDLR), there are more than 1700 mutations in the LDLR gene worldwide, reflecting the extensive genetic heterogeneity of FH patients, especially among different races. Researchers have speculated that the reason for this heterogeneity may be related to genetic drift, the founder effect, or intermarriage between FH patients. Large-scale clinical studies of FH have been conducted in many countries, but fewer data have been reported from China. Our group began to focus on FH in 2003 and has contributed to research on FH in China^{6–10}. Here, we provide a general overview of causative LDLR mutations in China, including the characteristics of the geographical distribution and phenotypes of patients with LDLR mutations and discussion of whether various other factors, such as the founder effect, are present.

Results

Study selection and research status of FH in China. Researchers reported the first Chinese case of FH in 1971¹¹. Subsequently, a number of FH patients were reported in Hong Kong, Taiwan Province, and Jiangsu Province, among others. A total of 1744 studies were analyzed for this review. After the removal of conference papers, duplicate articles and other unrelated studies, 353 potentially related studies were included in the analysis. In addition, three further studies were identified by reading reviews. Therefore, a total of 356 studies related to Chinese FH were selected. Of these, 74 studies that recorded mutations in FH were included in this systematic review (Fig. 1). A comprehensive literature analysis showed that the number of published articles on FH has increased each year, especially after 2004; 57.3% of the total articles were published after 2004 (Supplemental Fig. S1 online). However, more than half of the studies were case reports and reviews. In addition, many researchers reported a single pedigree with FH, and fewer than 23.6% of the studies performed functional experiments to investigate causative mutations.

LDLR mutations in mainland China, Hong Kong and Taiwan Province. *Overall results.* In 1985, Cai *et al.* identified the first five Chinese HoFH patients using a radio-labeled ligand assay, although the technology at that time could not be used to define the site of the mutation¹². Later, Hobbs and Sun *et al.* identified LDLR mutation sites and their functions using PCR and cDNA cloning technology, respectively, in patients with FH from Jiangsu Province^{13,14}. In our systematic review, we found 63 related studies that reported Chinese LDLR mutation sites. Thus far, 295 probands, including 131 LDLR mutations, have been reported (Supplemental Table S1 online); the geographical distribution of the characteristics of the LDLR mutations is shown in Supplemental Figure S2 online. The distribution of mutations revealed that most of the mutations were located in exon 4, and the next largest

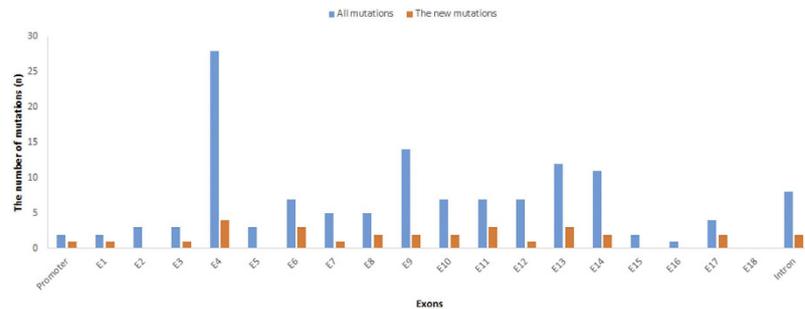


Figure 2. Distribution of Chinese LDLR mutations.

| Group* | Primary Mutations | | | | | Regional distribution in China | Also found in |
|---------|-------------------|-------------|----------------------|----------|--|---|--|
| | Exon | cDNA | Protein | Probands | Function | | |
| Group 1 | E7 | c.986G > A | p.C308Y (Cys329Tyr) | 26 | 31% LDLR activity in transfected COS cells | Beijing, Guangdong Province, Taiwan Province, Hong Kong | Russia ²⁰ , Philippines ²¹ , Hong Kong ²² , and the Netherlands ²³ |
| | E12 | c.1747C > T | p. H562Y (His583Tyr) | 23 | Precursor accumulates; approximately 50% mature protein | Jiangsu Province, Shanghai, Taiwan Province | Philippines ²¹ |
| | E13 | c.1879G > A | p. A606T (Ala627Thr) | 19 | bind LDL slow processing recycling defective | Anhui Province, Beijing, Henan Province, Hebei Province, Hubei Province, Jiangsu Province, Taiwan Province, Hong Kong | NA |
| Group 2 | E13 | c.1879G > A | p. A606T (Ala627Thr) | 13 | bind LDL slow processing recycling defective | Anhui Province, Beijing, Henan Province, Hebei Province, Hubei Province, Jiangsu Province | NA |
| | E10 | c.1448G > A | p. W462X (Trp483X) | 10 | 17% LDLR binding and 39% internalization activity in transfected 293T cells | Anhui Province, Guangdong Province, Hubei Province, Jiangsu Province, Zhejiang Province | Austria ⁴⁷ |
| | E13 | c.1864G > T | p. D601Y (Asp622Tyr) | 6 | 13.6% LDLR expression and 21.1% binding activity in peripheral blood lymphocytes from patients | Anhui Province, Beijing, Hubei Province, Shanxi Province | NA |

Table 1. Characteristics of the primary mutations in China. *Group 1 included Hong Kong, Taiwan Province and mainland China; Group 2 only included mainland China.

percentages of mutations were located in exons 9, 13, and 14. There was also a high number of intronic mutations, similar to a recently reported study¹⁵ (Fig. 2). In addition, approximately 60.3% (79/131) of the mutations were missense mutations, 13% (17/131) were nonsense mutations, and 2.3% (3/131) were large fragment deletion mutations. However, the functions of only 30.5% (40/131) of the mutations have been identified.

Among all of the 131 mutations, three mutations were reported more frequently: C308Y (c.986G > A, p.Cys329Tyr), H562Y (c.1747C > T, His583Tyr) and A606T (c.1879G > A, p.Ala627Thr) (Table 1), which accounted for 23% of the probands. Furthermore, three groups were identified based on their different geographical locations in China to determine whether there were characteristic mutations in each region: the North of China group, the South of China group, and the Taiwan Province group. Because of the special historical background of Taiwan Province, it was classified as an independent group. The three most common mutations in the North of China group were A606T (c.1879G > A, p.Ala627Thr), D601Y (c.1864G > T, p.Asp622Tyr) and 313 + 1G > A, and their frequencies represented 18.5%, 14.8%, and 7.4% of the probands in the North, respectively. The main mutations in the South of China group were not the same as in the North; they were W462X (c.1448G > A, p.Trp483X), A606T (c.1879G > A, p.Ala627Thr), and L393R (c.1241T > G, p.Leu414Arg), accounting for 10.7%, 7.5%, and 5.4%, respectively, of the probands in the South. However, there was a large difference between Taiwan Province and the other groups. The three most common mutations in Taiwan Province were C308Y (c.986G > A, p.Cys329Tyr), H562Y (c.1747C > T, His583Tyr), and D69N (c.268G > A, p.Asp90Asn), which accounted for 12%, 11.4%, and 7.4% of the Taiwanese probands, respectively (Fig. 3).

In addition, we identified 30 mutations that were not recorded in the above two LDLR databases (Table 2). The most common type of mutation was a missense mutation (63.3%); frame-shift mutations

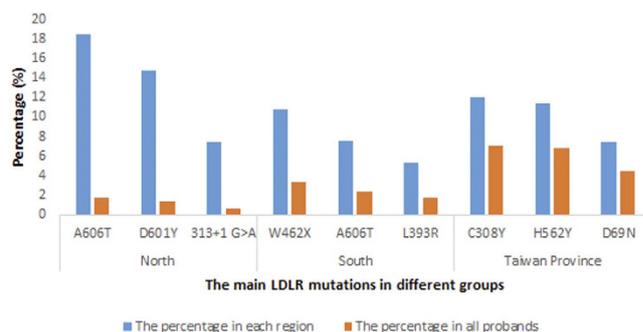


Figure 3. Frequencies of the main LDLR mutations in different regions of China.

accounted for 20% of the mutations. Functional studies have been performed for only 8 mutations. Therefore, the following open access software was used to predict the pathogenicity of the other mutations: (1) PolyPhen 2¹⁶, (2) SIFT¹⁷, and (3) Mutation Taster¹⁸. Because nonsense substitutions and frame-shift rearrangements are known to be pathogenic, we did not perform an *in silico* analysis for these mutations. A total of 13 mutations were analyzed, and most of the mutations were pathogenic, excluding two variants that were identified as non-pathogenic: N494S (c.1544A > G, p. Asn515Ser) and S533L (c.1661C > T, p. Ser554Leu).

FH in Taiwan Province. Chiou and Charng's group has been devoted to studying FH in Taiwan Province and has contributed greatly to the study of FH in the Chinese population. Since 2003, Charng has screened 170 unrelated hyperlipidemic Chinese patients using DNA screening and identified 10 mutations and two polymorphisms¹⁹. To date, a total of 175 probands, including 64 mutations, have been identified (Supplemental Table S1 online). Regarding the regional distribution, these probands could account for the highest percentage of probands in China, representing 59.3% of the total probands (Supplemental Fig. S2 online). There are three primary FH mutations in Taiwan Province. (1) C308Y (c.986G > A, p.Cys329Tyr) (21/175), in which the normal amino acid is a highly conserved cysteine residue at position 308. This variant may cause misfolding of the LDLR protein; the mutated protein localizes in the endoplasmic reticulum (ER) and exerts only 31% LDLR activity when transiently expressed in COS-7 cells¹⁹. However, this mutation has also been identified in Russia²⁰, the Philippines²¹, Hong Kong²², and the Netherlands²³. (2) H562Y (c.1747C > T, His583Tyr) (20/175) was first identified in a Chinese patient with FH by researchers from Jiangsu Province¹⁴. This mutation is located in the EGF-precursor domain, and as a result, only 50% of the mature protein is successfully synthesized. In addition, LDL binding is also limited when the mutated gene is transfected into CHO cells¹⁴. A haplotype analysis showed that the common ancestor of this mutation may have originated from an indigenous population (Yueh) living on the southeast coast of China, including Jiangsu, Guangdong and Fujian Provinces²⁴. (3) The D69N mutation (c.268G > A, p.Asp90Asn) (13/175) is located in a highly conserved region in the LDLR binding domain. In this mutation, the LDLR protein is retained in the ER and exerts only 55% of its normal activity. This mutation had also been identified in Hong Kong and Malaysia^{22,25}, and the results of a haplotype analysis suggested that it may have originated in southern China¹⁹.

FH in Hong Kong. Researchers first reported FH patients with mutations in Hong Kong in 1998²². The authors used single-strand conformation polymorphism (SSCP) and direct DNA sequencing to identify a total of 18 mutations in 30 Chinese patients with potential FH. In that study, three recurrent mutations were identified: L393R (3/21, c.1241T > G, p.Leu414Arg), C308Y (2/21, c.986G > A, p. Cys329Tyr) and V408M (2/21, c.1285G > A, p.Val429Met). The L393R mutation is located in exon 9 and may belong to class 5 because its functional domain is in the EGF spacer. The *in silico* analysis revealed that this mutation is likely to be pathogenic (Table 2). The V408M mutation is also present in exon 9 and was first reported in Afrikaans patients²⁶. A functional study in CHO cells showed < 2% LDLR activity in homozygotes. Unfortunately, there are no additional published studies on FH mutations in Hong Kong, and therefore, the above three recurrent mutations could not be implicated in common mutations due to the founder effect.

FH in mainland China. To date, 99 probands including 76 different mutations in 14 areas of mainland China have been reported (Supplemental Fig. S2 online). Regarding the regional distribution, the probands that originated from Guangdong Province have been reported most frequently, although the related reports are not from China^{25,27}. Other provinces that reported more than 8 probands included Jiangsu, Beijing, Anhui, Hubei, and Henan Provinces. Among all of the gene mutations, the most common ten mutations in mainland China are shown in Supplemental Figure S3A online. When the data

| Exon | cDNA | Protein | position | PhlyPhen-2 (Hum Div) | PhlyPhen-2 (Hum Var) | SIFT | Mutation Taster | Overall | LDLR Activity* |
|----------|---------------------|---------|----------|----------------------|----------------------|-----------|-----------------|----------------|--|
| E1 | C.-44C > T | | | | | | | | NA |
| E1 | 64delG | | 11089612 | | | | Disease causing | Pathogenic | NA |
| E3 | 310T > C | C83R | 11102783 | Probably Damaging | Probably Damaging | Damaging | Disease causing | Pathogenic | NA |
| E4 | c.383G > A | C107Y | 11105289 | Probably Damaging | Probably Damaging | Damaging | Disease causing | Pathogenic | NA |
| E4 | c.385G > T | D108Y | 11105291 | Probably Damaging | Probably Damaging | Damaging | Disease causing | Pathogenic | NA |
| E4 | c.444T > A | C127X | 11105350 | | | | Disease causing | Pathogenic | NA |
| E4 | 551_553delGTAAinsTT | | | | | | | Pathogenic | NA |
| E6 | c.889delA | | 11107463 | | | | Disease causing | Pathogenic | 14% LDLR activity in peripheral blood lymphocytes from HoFH patients |
| E6 | c.890A > C | N276T | 11107464 | Possibly Damaging | Possibly Damaging | Damaging | Disease causing | Pathogenic | NA |
| E6 | c.892delA | | 11107466 | | | | Disease causing | Pathogenic | NA |
| E7 | c.1054 T > A | C331S | 11110765 | Probably Damaging | Probably Damaging | Damaging | Disease causing | Pathogenic | NA |
| E8 | c.1100 T > A | L346H | 11111553 | Possibly Damaging | Possibly Damaging | Damaging | Disease causing | Pathogenic | NA |
| E8 | c.1129 T > G | C356G | 11111582 | | | | | Pathogenic | 57% LDLR binding and 52% internalization activity in transfected 293T cells |
| E9 | c.1304A > G | E414G | 11113395 | Probably Damaging | Probably Damaging | Damaging | Disease causing | Pathogenic | NA |
| E9 | c.1329delG | | 11113420 | | | | Disease causing | Pathogenic | NA |
| E10 | c.1439C > T | A459V | 11113615 | | | | | Pathogenic | 39% LDLR expression, 63% binding and 76% internalization activity in peripheral blood lymphocytes from HoFH patients |
| E10 | c.1544A > G | N494S | 11113720 | Bening | Bening | Tolerated | Polymorphism | Non-pathogenic | NA |
| Intron10 | c.1586 + 1 G > T | | | | | | | | NA |
| Intron10 | c.1586 + 5 G > C | | | | | | | | NA |
| E11 | c.1592T > A | M510K | 11116099 | | | | | Pathogenic | 31.6% LDLR binding and 36.2% internalization activity in transfected COS-7 cells |
| E11 | c.1597T > C | W512R | 11116104 | | | | | Pathogenic | 0-6% LDLR activity in transfected COS cells |
| E11 | c.1661C > T | S533L | 11116168 | Possibly Damaging | Bening | Tolerated | Polymorphism | Non-pathogenic | NA |
| E12 | c.1757C > A | S565X | 11116910 | | | | Disease causing | Pathogenic | 16% LDLR binding and 19% internalization activity in transfected 293T cells |
| E13 | c.1849A > G | K596E | 11120092 | Bening | Bening | Damaging | Disease causing | Pathogenic | NA |
| E13 | c.1864G > T | D601Y | 11120110 | | | | | Pathogenic | 13.6% LDLR expression and 21.1% binding activity in peripheral blood lymphocytes from patients |
| E13 | c.1877A > G | E605G | 11120123 | Possibly Damaging | Possibly Damaging | Damaging | Disease causing | Pathogenic | NA |
| E14 | c.2021A > G | N653S | 11120403 | Bening | Bening | Damaging | Disease causing | Pathogenic | NA |
| E14 | c.2075C > G | P671G | 1120457 | Probably Damaging | Probably Damaging | Damaging | Disease causing | Pathogenic | NA |
| E17 | 2400insG | | 11129563 | | | | Disease causing | Pathogenic | NA |
| E17 | c.2443C > T | L794F | 11129566 | | | | | Pathogenic | Normal LDLR binding, but 3% internalization activity in skin fibroblast from homozygous FH |

Table 2. Characteristics of mutations that were not recorded in LDLR databases and *in silico* predictions. All references are showed in supplemental table S1. *Only be reported in China.

obtained for Taiwan Province and Hong Kong are included in the analysis, the top ten most common mutations change (Supplemental Fig. S3B online). However, we do not discuss every mutation in each city because the numbers of probands in each city were not sufficient to confirm the origin of each mutation. Therefore, we analyzed only the three primary mutations in mainland China (Table 1).

The three primary mutations are A606T (c.1879G > A, p.Ala627Thr), W462X (c.1448G > A, p.Trp483X) and D601Y (c.1864G > T, p.Asp622Tyr), which account for 29.3% of probands in mainland China. (1) The first mutation, A606T, is located in the thirteenth exon encoding the EGF precursor homology domain. This mutation can lead to reductions in the mobility of the mature LDLR protein, precursor protein synthesis, LDL binding, and the rate of recirculation¹⁴. (2) The second mutation, W462X, includes a G-to-A change at nucleotide 1448 in exon 10 and was first reported in Jiangsu Province in China¹⁴. Our group discovered that the W462X mutation results in a truncated LDLR protein with only 17% LDL binding activity and 39% LDL internalization, despite a residual LDLR expression level of 81%⁷. (3) The third mutation, D601Y, is also located in the thirteenth exon and was first reported by our group in 2008 in China²⁸. Flow cytometric analyses of lymphocytes from HoFH patients revealed that the mutation is associated with LDLR expression of only 13.6%, LDL binding of 21.1% and LDL internalization of 94.3% in comparison to the controls²⁸.

Other mutations in FH in China. In addition to the LDLR mutations, other mutations can also cause FH, such as mutations in the apoB and PCSK9 genes. However, only 12 studies have reported apoB and PCSK9 mutations in China. The most common apoB mutation is R3500W (c.10707 C > T, 50/56), which is located in exon 26 and was first reported by Huang *et al.* in 1998²⁹. The other apoB mutations were R3500Q (E26, c.10708 G > A, 3/56), T3540M (E26, c.10828 C > T, 1/56) and R4019W (E29, c.12265 C > T, 2/56). Six mutations in PCSK9 have been reported: intron 2 T > G, R306S, V312S, V312F, R319E, and D320N (Supplemental Table S2 online). Functional experiments have been reported for only R306S, and the results revealed that mature LDLR was significantly decreased by 12% following transfection of the R306S mutant into Bel-7402 cells³⁰.

The clinical phenotypes of FH in China. The first study to discuss phenotypic variations in HeFH patients in China was published in 1998, in which Chinese patients with FH were compared with patients with the same or similar mutations in Canada²⁷. The study reported that the total cholesterol (TC) and LDL-C values in patients living in Canada were higher than those in patients living in China, similar to the incidence rate of coronary artery disease (CAD). These results suggested that environmental factors are very important for the clinical phenotype of FH patients. Later, researchers from Taiwan Province and Hong Kong reported the characteristics of FH patients in their cities^{31,32}. Their data showed that the TC levels in Taiwan Province (9.1 mmol/L) were similar to those in Hong Kong (9.4 mmol/L), higher than the levels in Japan (8.4 mmol/L)³³, Malaysia (7.7 mmol/L)³⁴, Australia (6.46 mmol/L)³⁵ and the Netherlands (5.97 mmol/L)³⁶, and lower than the levels in the UK (10.26 mmol/L)³⁷ and Spain (10.79 mmol/L)³⁸ (Table 3). The data obtained for HeFH patients in mainland China were selected from 1998 and showed that both the TC and LDL-C levels were lower compared with the other countries, excluding the Netherlands²⁷ (Table 3). Moreover, the higher xanthomata rates of FH patients in Japan and Malaysian were accompanied by a significant increase in CVD events. However, patients with FH in Australia and the Netherlands exhibited both lower TC and LDL-C values and a lower rate of CVD.

The relationship between mutations and clinical phenotypes. The data obtained for clinical features and lipid values in the included studies were also collected. A total of 524 patients were diagnosed with FH by gene screening, but clinical features or (and) lipid values were reported for only 355 patients. Table 4 shows the clinical characteristics of the different types of patients with FH. Among these data, we found that both HoFH and compound heterozygous FH patients were younger than the other groups, and they were also more vulnerable to corneal arcus, xanthoma and pCHD. Moreover, the levels of both TC and LDL-C were higher in HoFH than in the compound heterozygous, heterozygous and mutation-negative groups. Interestingly, the heterozygous patients had lower TC and LDL-C levels compared with the mutation-negative group. In addition, 6 patients showed a clinical homozygous phenotype but were genetically heterozygous and presented higher TC and LDL-C values compared with homozygous patients (Table 4). We also evaluated the clinical characteristics associated with different types of gene mutations (Supplemental Table S3 online). Clearly, the TC and LDL-C levels of HoFH patients were higher than those of HeFH patients in each group. Moreover, frameshift mutations appeared to be associated with higher TC levels in either HoFH or HeFH patients compared with the other groups.

The diagnostic criteria for FH and screening methods in China. There are three internationally recognized clinical diagnostic criteria for FH worldwide: the DLCN criteria, the “Simon Broome” UK-FH register criteria, and the MEDPED System¹. Scholars in Hong Kong and Taiwan Province have adopted one of the above clinical diagnostic criteria for FH, whereas in mainland China, scholars use different clinical diagnostic criteria based on the book “Clinical coronary heart disease”, which was published in

| Countries | TC(mmol/L) | LDL-C (mmol/L) | TG(mmol/L) | HDL-C (mmol/L) | Xanthomata (%) | Corneal arcus(%) | CVD(%) |
|-------------------------------------|------------|----------------|------------|----------------|----------------|------------------|--------|
| Taiwan Province ³¹ | 9.1 | 6.83 | 1.31 | 1.45 | 23.5 | NA | 24.5 |
| Hong Kong ³² | 9.41 | 7.49 | 1.3 | 1.35 | 50 | 49.1 | 13 |
| Japan ³³ | 8.4 | 6.4 | 1.49 | 1.22 | 87 | 38 | 24 |
| Malaysian ³⁴ | 7.7 | 5.1 | 1.9 | 1.3 | 50.6 | 50.6 | 80.5 |
| Australia ³⁵ | 6.46 | 4.42 | 1.2 | 1.3 | 18.6 | 26.1 | 9.6 |
| Netherlands ³⁶ | 5.97 | 4.13 | 1.1 | 1.2 | NA | NA | 9.2 |
| UK ³⁷ | 10.26 | 4.69* | 1.34* | 1.36* | 28.6 | 16.7 | 19.4 |
| Spain ³⁸ | 10.79 | 8.68 | 1.2 | 1.34 | 28.5 | NA | 21.9 |
| Mainland China (1998) ²⁷ | 6.09 | 4.35 | 1.23 | 1.32 | 0 | NA | 0 |

Table 3. Clinical features and blood lipid values of FH in different countries. All lipid value were used as average value in each studies. CVD, cardiovascular disease; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; TC, total cholesterol; TG, triglyceride. *Treated lipid values.

| | Homozygous FH | Compound Heterozygous FH | Heterozygous FH | Mutation (–) | Heterozygous but Clinical Homozygous FH* |
|------------------------|----------------------|--------------------------|-----------------------|-----------------------|--|
| Numbers | 42 | 19 | 285 | 18 | 6 |
| Ages | 18.3 ± 14.9 | 13 ± 11 | 39.9 ± 16.3 | 35.8 ± 13.8 | 18.8 ± 18.5 |
| Male (%) | 45.2% | 57.9% | 48.4% | 66.7% | 50% |
| Corneal arcus (%, n/N) | 76.5% (13/17) | 57.1% (4/7) | 34.8% (23/66) | 11.1% (1/9) | 0 (1) |
| Xanthoma (%, n/N) | 95% (38/40) | 100% (19/19) | 31.6% (54/171) | 50% (9/18) | 100% (5/5) |
| CVD (%, n/N) | 57.1% (20/35) | 57.9% (11/19) | 22.2% (37/167) | 11.1% (2/18) | 20% (1/5) |
| TC (mmol/L, n/N) | 17.47 ± 4.2 (42/42) | 15.87 ± 4.27 (19/19) | 8.21 ± 2.02 (285/285) | 9.47 ± 4.06 (18/18) | 18.03 ± 4.75 (6/6) |
| LDL-C (mmol/L, n/N) | 15.02 ± 4.63 (38/42) | 13.07 ± 3.29 (19/19) | 6.17 ± 1.91 (245/285) | 7.83 ± 4.12 (18/18) | 15.93 ± 3.6 (6/6) |
| TG (mmol/L, n/N) | 1.3 ± 0.67 (34/42) | 1.25 ± 0.63 (17/19) | 1.53 ± 1.07 (239/285) | 1.24 ± 0.45 (18/18) | 1.61 ± 0.72 (5/6) |
| HDL (mmol/L, n/N) | 1.14 ± 0.42 (36/42) | 1.28 ± 0.63 (19/19) | 1.37 ± 0.69 (223/285) | 1.16 ± ± 0.32 (18/18) | 0.65 ± 0.27 (5/6) |

Table 4. Relationship between mutations and clinical phenotypes. n: the number of patients with recorded information; N: all the patients of each group. *this group included patients which showed clinical homozygous phenotype but were genetically heterozygous.

1998³⁹. If patients and/or their relatives have tendon xanthoma and meet one of the following conditions, they may be diagnosed with FH: 1. child <16 years of age with plasma TC levels >6.7 mmol/L (300 mg/dl); 2. adult with plasma TC levels >7.8 mmol/L (260 mg/dl); or 3. patient with plasma LDL-C values >4.9 mmol/L (190 mg/dl). When patients with tendon xanthoma have a TC level >16 mmol/L (60 mg/dl), they are diagnosed as HoFH; otherwise, they may be diagnosed as HeFH.

The gene diagnoses are also important. The Chinese researcher Sun first used SSCP and Southern blotting to detect LDLR mutations in Chinese patients in 1994, but all of the experiments were completed in the UK¹⁴. Other technologies have also been employed, such as long-chain polymerase chain reaction⁴⁰ and polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP)⁴¹. Although these methods can detect whether variations are present, they are not capable of confirming the sites of the mutations. In 2001, Wang *et al.* published an SSCP and DNA sequencing analysis to screen all 18 exons and exon-intron boundaries in the LDLR gene. The authors identified one mutation in exon 6 (c.850 T > C, p. Cys284Arg)⁴². Our group later conducted touch-down PCR together with DNA sequencing and denaturing high-performance liquid chromatography (DHPLC) to detect LDLR mutations^{6,43}. We also conducted the first study to utilize an SNP-based genome-wide linkage scan and whole/targeted exome sequencing technologies to detect LDLR mutations in China^{8,10,44}.

Discussion

In this systematic review, we analyzed the characteristics, distribution, gene frequency, and relationship between the genotype and phenotype of LDLR gene mutations in the Chinese population. A total of 74

studies, involving 586 patients (including 357 probands) with 141 related mutations in FH, were included in this review. In summary, the findings from these studies indicate that although FH is widely distributed in most regions of China, the diagnostic and therapeutic rates of FH were less than 1%. This finding suggests that most doctors and people lack an understanding of FH and also pay insufficient attention to the occurrence of FH in China.

To date, only one study has estimated the prevalence and treatment of FH, which included 9,324 Chinese subjects from the Jiangsu Nutrition Study. The researchers reported that the prevalence of probable/definite FH was 0.28% (1.4/500) based on the modified DLCN definition, only 15.9% of the patients were receiving drug treatment, and none of the patients achieved the LDL target value (<2.5 mmol/L)⁴. This study also demonstrated that FH patients had more than a 15-fold increased risk of cardiovascular disease compared with patients without FH. The authors suggested that FH is not rare in the Chinese population and remains largely unidentified. Chiou reported that the frequency of apoB gene mutations is less than 10% in Taiwan Province²⁴. In addition, the frequency of PCSK9 gene mutations appeared to be less than 5% because Song *et al.* identified only 5 mutations in 100 hyperlipidemia patients and did not detect any LDLR or apoB mutations⁴⁵. However, a large-scale clinical study for FH screening in mainland China should be performed to confirm these results.

It is well known that high-frequency LDLR gene mutations can aid in the screening for FH. For example, the S472RfsX44 mutation (in exon 10) is responsible for approximately 42% of the patients with FH in Tunisia⁴⁶. The nine most common mutations have been shown to be responsible for 66.5% of the patients with FH in the Netherlands²³. Our review revealed that the five most common mutations (C308Y, H562Y, A606T, D69N, and W462X) could explain 31.9% of the FH probands in China (Supplemental Fig. 3 online). Furthermore, the three most common mutations in mainland China encompass 43.8% of the provinces with reported mutations, and these mutations have only been reported in the Chinese population, excluding the W462X mutation, which is also found in Australia⁴⁷ (Supplemental Fig. 3 online). This finding suggests that the above mutations may be the most specific and common mutations in China, which may be useful for FH screening in the future. However, owing to the small sample size, future large-scale screening trials should be conducted to confirm these conclusions.

In the analysis of clinical phenotypes, we discovered that levels of TC and LDL-C in patients from Hong Kong and Taiwan Province were similar to the levels measured in patients in Western countries and higher than those in patients in Asia and parts of Europe. One possible explanation for this finding is that the diets and lifestyles of these patients are similar to those of patients in Western countries. In analyses of the relationship between mutations and clinical phenotypes, we discovered two interesting phenomena: (1) the mutation-negative group had lipid values that were comparable to the HeFH group and (2) there were 6 probands that were diagnosed as having a HeFH genotype but actually had a clinical homozygous phenotype. These data suggest that there may be other pathogenic genes or many minor genes that participate in the development of disease, as suggested in a previous study⁴⁸. Therefore, future studies should be conducted to confirm these phenomena.

There are several limitations to this review. First, different nomenclatures were utilized for the LDLR mutation in the included studies. Hence, we unified the nomenclature of the mutations in this review. Second, some of the studies did not mention the origin of the probands. Consequently, we used the location of the first author affiliation. Thus, there are some deviations in the regional distribution of the mutations. Third, we reviewed and analyzed the clinical data obtained for the FH patients. However, the conclusions from these data analyses should be treated with caution because this was a systematic review, and all the data were analyzed in a secondary manner. Finally, we included only LDLR mutations identified in China, which may result in regional limitations.

Conclusions

In recent years, interest in FH has grown among the international community, and many countries have published FH guidelines to facilitate improved management and treatment of patients with FH^{1,3}. The current review identified only 357 probands in China, including 131 LDLR mutations, 4 apoB mutations, and 6 PCSK9 mutations, which may provide information that is specific to China for inclusion in the international FH database. However, the functions of these mutations and which mutations influence the prognosis of patients remain unknown. Furthermore, whether other genes or SNPs exist that may cause this disease or influence the treatment of FH remains to be discovered. These questions should be addressed by researchers in China in future studies. However, a reliance on doctors and laboratory personnel to address FH is insufficient, and it will be critical to raise public awareness of FH. Finally, China is one of the most populous countries in the world and may have a greater genetic burden resulting in more patients with FH. We hope that the present review will raise awareness among doctors and the public and promote the initiation of future studies to explore the diagnosis and treatment of FH.

Methods

Search strategy. Using the computerized literature databases of PubMed, Embase, Wanfang (Chinese), the Chinese National Knowledge Infrastructure (CNKI, Chinese), and the Chinese Biological and Medical database (CBM, Chinese), and public data were limited to December 2014. The Medical Subject Heading terms and the following key words were used: “familial hypercholesterolemia”, “Chinese”,

“China”, “Hong Kong”, and “Taiwan”. Only English and Chinese language articles were included in the analysis.

Selection and definition of gene nomenclature. The inclusion criteria for the included studies were as follows: any reports of pathogenic mutations in FH, such as LDLR, APOB, and PCSK9 gene mutations. Two nomenclatures were used to describe LDLR gene mutations in the LOVD databases. The first nomenclature was based on the guidelines from www.hgvs.org/mutnomen, starting with nucleotide + 1 as the A in the ATG translation initiation codon⁴⁹. The second nomenclature begins with negative numbers from the exon 1 codons⁵⁰. In this review, we adopted the second nomenclature as the primary form and listed the alternate nomenclature in brackets.

Data extraction. Two independent researchers (L.Y.S. and Y.F.D.) reviewed the relative articles based on the abstracts and full texts to assess their eligibility. One researcher (E.Z.) collected information concerning the LDLR mutations, including the exons, cDNA, proteins, distribution, and function of the LDLR gene mutations. If the articles reported sequencing information, then the information for mutations was assessed between the images and original nucleotide sequence. If the information differed between the images and text describing the mutations, we collected information regarding mutations from the sequencing results. The clinical characteristics included age, sex, and lipid values, among others. All authors of this review were consulted if additional data were collected or the data were unclear.

Mutation prediction. Novel LDLR variants were assessed by *in silico* mutation prediction tools, including PolyPhen2¹⁶, SIFT¹⁷, and Mutation Taster¹⁸.

Statistical analysis. All of the statistical analyses were performed using SPSS version 18.0 (SPSS, Inc., Chicago, Illinois). Measurement data are presented as the mean \pm SD, and count data are presented as rates.

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Author Contributions

L.J. wrote the main manuscript text and analyzed the data; L.Y.S. and Y.F.D. conducted the searches; S.W.Y. prepared the figures; F.Z. collected the data; and L.Y.W. supervised the work. All of the authors reviewed the manuscript.

Additional Information

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