Genetic screening for familial hypercholesterolaemia in Hong Kong

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KEY MESSAGES

1. Cascade screening of family members of known index cases is an effective approach to identify new cases of familial hypercholesterolaemia (FH).
2. Family screening is carried out using a combination of plasma lipid profiling and genetic testing. If the causative mutation is unknown or if genetic testing is unavailable, screening can be performed using plasma lipid profiling alone.
3. Over 90% of the FH subjects with a pre-treatment low-density lipoprotein cholesterol level of >8 mmol/L had an identifiable genetic cause.
4. Causative mutations in most FH patients were found in the low-density lipoprotein receptor gene.

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Introduction

Familial hypercholesterolaemia (FH) is an inherited disorder of lipid metabolism that results in high levels of low-density lipoprotein cholesterol (LDL-C) and increased risk of premature cardiovascular disease. The major genetic causes are mutations in the LDL-receptor (*LDLR*) gene, the apolipoprotein B-100 (*APOB*) gene, and/or the proprotein convertase subtilisin/kexin type 9 (*PCSK9*) gene. Recent studies suggest that heterozygous FH is more common than previously thought and affects approximately 1 in 200 to 250 persons in western countries. Early diagnosis and treatment with statin significantly improves the prognosis and reduces the cardiovascular burden.

Diagnosis of FH is mainly based on lipid levels and family history. Clinical diagnostic tools have been developed to standardise the phenotypic diagnosis of FH. The most widely used scoring systems are the Dutch Lipid Clinic Network (DLCN) Criteria and the Simon Broome Criteria.[1] These systems have various predictive values depending on the test population but have inherent limitations in sensitivity and specificity. Recent guidelines recommend that all patients with clinical and biochemical features of FH undergo DNA testing to confirm the diagnosis, and genetic cascade family screening enables unambiguous identification of affected relatives.[2,3]

Genetic testing for FH is not available in the public health care system in Hong Kong so the diagnosis of FH is based on clinical evidence. Family screening is performed only on an ad-hoc basis because of limited staffing and resources. It is likely that a considerable proportion of patients with FH remain undiagnosed. This study aimed to implement systematic cascade genetic screening of FH patients and their families. The study was expected to provide information about the spectrum of genetic mutations in Hong Kong Chinese patients with FH.

Methods

Index patients with a clinical diagnosis of FH and/or severe hypercholesterolaemia were recruited from the Queen Mary Hospital, Ruttonjee Hospital, and Pamela Youde Nethersole Eastern Hospital. The diagnosis of FH was classified as definite, probable, or possible according to the DLCN Criteria. Genetic analysis was carried out in index cases, and first-degree relatives were invited to attend cascade screening to detect potential new cases based on genetic testing (when a family-specific causative variant had been identified) or from phenotypic diagnostics. The newly confirmed cases were considered new index cases and their first-degree relatives were subsequently screened whenever possible.

Genomic DNA was isolated from peripheral blood leukocytes, and mutations were identified by Sanger sequencing of the coding regions of *LDLR*, *APOB*, or *PCSK9* genes. Exons and their adjacent introns, 5'-UTR and 3'-UTR, were amplified by polymerase chain reaction, and standard sequencing in both forward and reverse directions was performed using the 3730xl DNA Analyzer (Applied Biosystems, Foster City [CA], United States) at the Centre for Genomic Sciences of The

University of Hong Kong. Pathogenicity of identified variants was assessed by reference to published data in the following databases: the University College London FH mutation database (http://www.ucl.ac.uk/ldlr/Current/), the Human Gene Mutation Database (http://www.biobase2international.com/product/hgmd), and Ensembl GRCh38. Pathological assessment of novel variants was performed using multiple online tools.

TABLE 1. Clinical characteristics and pre-treatment lipid levels of index familial hypercholesterolaemia patients

Characteristic	Male (n=42)*	Female (n=52)*
Age, y	50.2±11.9	53.2±10.3
Weight, kg	70.3±9.5	58.9±8.4
Body mass index, kg/m^2	24.6±5.7	24.8±3.2
Cardiovascular disease	43%	40%
Total cholesterol, mmol/L	9.3±1.9	9.7±2.3
Total triglycerides, mmol/L	1.6±0.8	1.9±0.8
Low-density lipoprotein cholesterol, mmol/L	7.6±1.9	7.8±2.2
High-density lipoprotein cholesterol, mmol/L	1.2±0.4	1.4±0.5
Xanthoma/xanthelaesma at diagnosis	21%	15%

* Data are presented as mean ± standard deviation or % of patients

TABLE 2. Types of mutations in familial hypercholesterolaemia index patients

Types of mutations	Total No.	Low-density lipoprotein cholesterol, mmol/L*	No. of unique mutations	No. of novel mutations
Single mutation				
LDLR (total)	51	8.0±1.5	29	10
Splicing	2	9.0±0.4	1	0
Frameshift	2	8.2±1.3	2	1
Nonsense	4	8.5±1.6	3	1
Missense	43	7.9±1.6	23	8
APOB (missense)	3	7.3±1.1	2	0
PCSK9	0	-	0	0
Two mutations				
LDLR + *LDLR*	5	10.4±4.5	4	2
LDLR + *APOB*	3	9.6±3.0	3	0

* Data are presented as mean ± standard deviation

TABLE 3. Proportions of mutation-positive patients according to low-density lipoprotein cholesterol level

Low-density lipoprotein cholesterol, mmol/L	Total No.	Proportion with mutation (%)
<6	18	39
6-6.9	16	44
7-7.9	22	68
8-8.9	16	94
9-9.9	15	93
>10	7	71

Results

A total of 98 index patients were recruited and 94 probands were identified. Four patients were found to be related (Table 1). All but two patients were taking statins and 55 patients were taking two or more lipid-lowering agents, and two were receiving plasmapheresis. Only 20 patients who were undergoing treatment had an LDL-C level of <2.6 mmol/L, and seven of these patients had an LDL-C level of <1.8 mmol/L.

Genetic analysis was carried out in the 94 unrelated probands. Definite or likely pathogenic mutations were identified in 62 patients (Table 2). Most mutation-positive patients had a heterozygous *LDLR* gene mutation. There were five cases of compound heterozygous mutation, three cases of double heterozygous mutation, and one case of homozygous mutation of c.1474G>A in exon 10. Overall, mutation-positive patients had a significantly higher mean ± standard deviation LDL-C level at diagnosis than mutation-negative patients (8.1±1.9 vs 6.4±1.4 mmol/L, P<0.01). There was a stepwise increase in the proportion of patients with causative mutations identified according to the LDL-C stratum (Table 3). Of patients with an LDL-C level of >8 mmol/L, 90% were mutation-positive.

As genetic testing is not available in the public health care system in Hong Kong, diagnosis of FH is based on phenotypic criteria. We compared the diagnostic performance of the DLCN Criteria with the modified DLCN Criteria for Chinese, which uses lower LDL-C cut-offs based on population data derived in China.[4] On validation against genetic testing, the DLCN Criteria demonstrated the best overall performance in diagnosing FH (82.8% sensitivity, 53.3% specificity, 79.1% positive predictive value, and 59.3% negative predictive value). The modified DLCN Criteria had high sensitivity (93.8%) but low specificity (26.7%). The Simon Broome Criteria had lower sensitivity (64.0%) and higher specificity (56.6%), compared with the DLCN Criteria.

Cascade family screening was offered to all probands. Relatives of 45 of the 62 probands with causative mutations identified (167 first- and second-degree relatives) attended for screening. In contrast, relatives of only 13 of 32 probands with no mutations were identified (36 first-degree relatives) attended for screening. Only adults were screened, as screening for children and adolescents has not been approved. Of 203 relatives who attended for screening, 122 were identified to have FH: 48

were newly diagnosed and 74 were aware of having hypercholesterolaemia, although 53% of the latter had never been treated or had stopped treatment. The diagnosis of FH was excluded in 81 patients. In family members identified to have FH, 81% were diagnosed based on the finding of a causative mutation on genetic testing. The remaining were diagnosed based on clinical phenotype in their pedigrees when genetic testing did not detect a causative mutation.

Discussion

Our findings are similar to those previously reported in Chinese and Caucasian populations—namely, that the causative mutations in most FH patients are in the *LDLR* gene. Although the prevalence varies in different populations, heterozygous *LDLR*, *APOB*, and *PCSK9* mutations are found in >90%, ~5%, ~1%, respectively, of heterozygous FH patients with a causative mutation. We identified one homozygous FH patient with the mutation c.1474G>A in exon 10 of *LDLR*. This mutation causes a defective protein and leads to reduced function of *LDLR* rather than a null mutation.[5] We also identified a number of compound heterozygotes and double heterozygotes. These individuals have higher LDL-C levels, and such genotypes have been reported in some cases of phenotypic homozygous FH.

No genetic cause was identified in 34% of our FH patients, consistent with the proportion reported in the literature. This may be due to a number of reasons. We did not investigate rare mutations in minor genes, such as *APOE*, *ABCG5*, *ABCG8*, *LIPA*, or *STAP1*, which can phenotypically resemble FH. There may have been undiscovered monogenic causes of hypercholesterolaemia in some patients, and some patients may have had a polygenic cause and carried a disproportionately high burden of multiple small-effect common variants that raised the plasma LDL-C level. Other possible causes include epigenetic effects and interaction of environmental factors with unknown genetic determinants.

LDL-C is the most discriminating factor in the diagnosis of FH. Sensitivity and specificity of any scoring criteria can be varied by changing the LDL-C cut-off value. Our data suggest that LDL-C cut-offs in the DLCN Criteria can be applied to the Hong Kong population to diagnose FH and select patients for genetic testing. For genetic testing based on LDL-C levels alone, our data show a cut-off of 8 mmol/L, as 90% of the index subjects with a LDL-C level of >8 mmol/L had an identifiable genetic cause.

The most cost-effective approach to identify new FH patients is cascade screening of family members of known index cases. Having an identifiable genetic cause increases the likelihood of family members agreeing to participate in screening. Overall, the awareness of FH in family members is low in Hong Kong and the high risk of cardiovascular disease secondary to FH is poorly recognised. Among the relatives of FH patients who knew they had a high cholesterol level from previous lipid testing, half had never been treated or had discontinued treatment, especially those with a milder phenotype. There is a need to increase awareness and understanding of the condition to better prevent the development of cardiovascular disease in these patients.

Limitations

Our study has several limitations. We did not investigate rare mutations in minor genes, and we did not evaluate the potential contribution of polygenic effects. Pathological assessment of novel variants was based on in silico analysis only; further functional studies are needed to confirm the pathogenic effects. Only adults were screened; screening of relatives younger than 18 years was restricted.

Conclusions

In clinically ascertained patients with FH and/or severe hypercholesterolaemia, about two-thirds had a discrete genetic basis of disease, with most causative mutations occurring in the *LDLR* gene. Genetic cascade screening is feasible. Having an identifiable genetic cause increases the likelihood of family members agreeing to participate in screening.

Familial hypercholesterolaemia is underdiagnosed and undertreated in Hong Kong. Awareness of the condition in relatives of affected individuals and in the community is low, and education of the public and health care professionals is needed. Cascade screening of family members is an effective means to identify new FH cases. Screening can be carried out using a combination of plasma lipid profiling and genetic testing. If the causative mutation is unknown or genetic testing is unavailable, screening can be performed using plasma lipid profiling alone. Data collected in this study may help formulate future health care policies on the eligibility of FH patients for novel therapies such as PCSK9 inhibitors. About 90% of our FH patients with a LDL-C level of >8 mmol/L had an identifiable genetic cause. These patients with severe FH are unlikely to achieve the recommended LDL-C target with current oral lipid-lowering agents. They are candidates for PCSK9 inhibitors if funding can be obtained.

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Ethical Approval

The study was approved by the Institutional Review

Board of The University of Hong Kong / Hospital Authority Hong Kong West Cluster and Hong Kong East Cluster Research Ethics Committee (UW 12-494 and HKEC-2013-005, respectively). Informed consent was obtained from each participant.

Declaration

The authors have no conflict of interest to disclose.

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