Mutations in the Low-Density Lipoprotein Receptor Gene in Chinese Familial Hypercholesterolemia Patients

Ying-Tat Mak, Chi-Pui Pang, Brian Tomlinson, Jun Zhang, Yuen-Shan Chan, Tony W.L. Mak, John R.L. Masarei

Abstract—It has been reported that in China, patients with heterozygous familial hypercholesterolemia (FH) may go unrecognized because they do not have xanthomata or premature coronary heart disease and their LDL cholesterol levels are lower than those in their Western counterparts. However, in the Chinese patients in Hong Kong, heterozygous FH appears to manifest in a way similar to that seen in Western countries or Japan. We studied sequence variations in the promoter and coding regions of the 18 exons of the LDL receptor gene in 30 Chinese FH patients. Eighteen mutations were identified in 21 patients scattered in the promoter and 10 exons. Eleven of them were first found in this study. We also found 6 polymorphisms with allelic frequencies different from those in whites but similar to the Japanese, indicating some isolation between white and Oriental populations. A total of 29 mutations in the LDL receptor gene are now known in the Chinese. There is no definite common mutation due to a founder effect. Meanwhile, there were no detectable LDL receptor gene mutations in 9 clinically diagnosed FH patients in whom the R3500Q mutation in apolipoprotein B had also been excluded. The gene defects leading to the FH phenotype in these patients may occur somewhere else in the apolipoprotein B or other related genes, or even in the noncoding sequences of the LDL receptor gene. (Arterioscler Thromb Vasc Biol. 1998;18:1600-1605.)

Key Words: Chinese ■ familial hypercholesterolemia ■ LDL receptor gene

The LDL receptor is a cell-surface glycoprotein that regulates the plasma cholesterol level by specific uptake of LDL particles from the blood circulation.1 The LDL receptor gene, located at chromosome 19p13.1–3, consists of 18 exons spanning 45 kb and codes for a 5.3-kb mRNA.2 Translation of 2.5 kb of the mRNA produces a receptor protein of 839 amino acids.3 Defects in the LDL receptor gene are responsible for familial hypercholesterolemia (FH). There are more than 200 reported mutations scattered over the entire gene.2 In a few populations, such as the Afrikaners and Finnish, founder mutations account for the majority of mutations in their FH patients. In most populations around the world, the LDL receptor gene mutations are heterogeneous.2

FH has a prevalence of about 1 in 500 in most populations and is inherited in an autosomal-dominant mode. Homozygous FH occurs in about 1 in 1 million and usually leads to premature death due to coronary heart disease (CHD). Heterozygous FH is probably the most common monogenic disorder, with preventable consequences and effective treatments.5 The diagnosis of FH is based mainly on family history, plasma total and LDL cholesterol levels, the presence of tendon xanthomata and premature CHD, or similar findings in first-degree relatives.5 However, up to 15% of FH subjects, especially children, might be misdiagnosed using these criteria.4 An alternative diagnostic approach is cellular assay of the ability of lymphocytes or fibroblasts to bind or internalize LDL, which is technically tedious and not suitable for routine clinical use.5 Direct DNA analysis should provide an unequivocal result and allow appropriate early genotype-specific treatment as well as antenatal and family studies.6 Polymerase chain reaction (PCR) and single-strand conformation polymorphism (SSCP) are efficient and effective methods for detecting mutations in the LDL receptor gene.2,7,8 The prevalence of FH in whites and Asians is probably similar.9 Although the prevalence has not been documented in the Chinese, clinical experience has indicated that heterozygous FH is a common disorder leading to atherosclerosis and CHD.10 Studies in China suggest that patients with heterozygous FH may lack the usual clinical expression and thus may not be identified unless they are a relative of a homozygote.11 It was suggested this factor may be due to the traditional low-fat Chinese diet, resulting in low levels of LDL cholesterol, or to genetic influences such as a “cholesterol-lowering” gene. Our experience in Hong Kong, where the diet is higher in fat and population cholesterol levels are more similar to Western countries, suggests that heterozygous FH patients show similar clinical expression to those in the West or Japan.9 To date, only 12 mutations in the LDL receptor gene have been reported in the Chinese.11 Whether common mutations of diagnostic value exist in the LDL receptor gene

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in the Chinese is uncertain. Here we report our investigation of the promoter and 18 coding exons of the LDL receptor gene for mutations in Chinese FH patients.

Methods

Patients

Chinese patients attending the Lipid Clinic, Prince of Wales Hospital, diagnosed as FH heterozygotes or homozygotes were recruited into the study. The diagnostic criteria included (1) fasting plasma total cholesterol levels >7.5 mmol/L in adults (>6.5 mmol/L in individuals younger than 16 years) and having normal fasting plasma triglyceride levels, and (2) presence of tendon xanthomata in proband or first degree relative, or other family members having raised LDL cholesterol inherited in a dominant pattern. Family members of the probands were invited for investigation of FH. The occurrence of familial defective apolipoprotein B-100 due to the R3500Q mutation was detected by a mutagenic PCR method.12

PCR

The promoter and 18 coding exons of the LDL receptor gene were amplified by PCR using primers as described.2 Genomic DNA was extracted from EDTA whole blood samples by the salting-out method.13 Each PCR mixture, 25 μL in volume, contained 50 ng DNA, 100 μmol/L each dNTP, 15 pmol of each primer, 2.0 mmol/L magnesium chloride, and 0.5 U Taq DNA polymerase (GIBCO-BRL). The PCR was carried out on a TC1 thermal cycler (Perkin Elmer-Cetus): 35 cycles of 95°C for 45 seconds and 68°C for 6 minutes, final extension at 68°C for 15 minutes. For SSCP analysis, 0.1 μL of [α-32P]dCTP (Amersham) was added to the reaction mixture prior to PCR.

SSCP

The [α-32P]-labeled PCR product (1 μL) was mixed with 10 μL of loading dye (95% formamide, 10 mmol/L EDTA, 0.1% bromophenol blue, and 0.1% xylene cyanol) and denatured at 95°C for 5 minutes. After snap-cooling in ice, 6 μL of each mixture was electrophoresed in 6% polyacrylamide gel (acrylamide: bisacrylamide = 49:1) with or without 10% glycerol in 45 mmol/L borate buffer pH 8.3 at 4°C and 68°C for 6°C. The 18 PCR products were sequenced using a double-strand DNA cycle sequencing kit (GIBCO-BRL) after ammonium acetate and isopropanol precipitation. The primers for PCR were used after being end-labeled with [α-32P]ATP (Amersham). The same PCR temperature program was used for the sequencing reaction, but the cycle number was 30.

Haplotyping of the LDL Receptor Gene

The haplotypes of the LDL receptor gene were analyzed using 4 polymorphic sites: SfaN in exon 2, AvaII in exon 13, NcoI in exon 18, and TA repeats in the 3′-untranslated region of exon 18.15,16 All polymorphic sites were confirmed by direct double-strand DNA sequencing.

Results

A total of 30 unrelated FH patients were recruited. Demographic details, clinical features, and lipid levels are shown in Table 1. The plasma total cholesterol levels of these patients ranged from 7.4 to 16.0 mmol/L and LDL cholesterol from 5.7 to 14.3 mmol/L when on no lipid-lowering drug therapy. Nucleotide changes in the LDL receptor gene were detected by SSCP in 21 of them. Direct DNA sequencing of the PCR products identified 18 mutations (Table 2). For the 21 subjects with mutations, a total of 69 first-degree relatives were available for testing. Of these 69 relatives, 45 were found to have the same mutation as the proband of their family. Their ages ranged from 5 to 84 years and their total cholesterol levels from 6.8 to 13.3 mmol/L. There were 24 first-degree relatives in whom the mutations were not identified. Their ages ranged from 8 to 76 years and their total cholesterol levels from 3.3 to 6.8 mmol/L.

Apart from subject L322, who was a compound heterozygote of the −44C→T and P604L mutations, all subjects were heterozygous for the mutations (Table 2). L322 had coronary artery bypass graft surgery at the age of 27. His plasma cholesterol level was around 12 mmol/L despite treatment including plasmapheresis. The P664L mutation was inherited from his father, but his mother does not carry either mutation (Figure 1).

In proband L24 with the mutation V776M, the G→A substitution at nucleotide 2389 occurs at the last base of exon 16, which may be the −1 position of the 5′ donor splice site. Its effect on splicing was investigated by reverse transcription–PCR. Only the normal G allele was found. This mutation thus appears to cause a donor site splicing error, the first of such a mutation reported for the LDL receptor gene.

No mutation was detected in 9 patients in the promoter and all the known coding regions of the LDL receptor gene. From these 9 probands, there were 23 first-degree relatives available for testing. No mutations were found for these regions of the LDL-receptor gene in the relatives. Their ages ranged from 17 to 62 and their total cholesterol levels from 3.8 to 12.9 mmol/L. The hypercholesterolemia appeared to show bimodal inheritance, as demonstrated in 2 of the family pedigrees shown in Figure 2.

Familial defective apolipoprotein B was not found by DNA analysis of the R3500Q mutation in any study subjects.

In our SSCP analysis, the presence of glycerol in the electrophoresis gel was crucial. Without glycerol, no SSCP could be detected in any of the PCR products. With 10% glycerol, SSCP was obtained in 22 PCR products of 21 patients. Of these PCR products, 4 were obtained from electrophoresis at 4°C and 18 at room temperature. The 18 mutations were identified in these 22 PCR products, since some patients shared the same mutations and 1 patient, L322, has 2 mutations. A 100% sensitivity of our SSCP analysis, confirmed by sequencing all the PCR products of all study subjects.
were first found in this study (Table 2). They were scattered over the LDL receptor gene and changed conserved amino acid sequences in different functional domains of the mature protein. When family members were available for study, the mutations were shown to cosegregate with and therefore presumably responsible for the FH. In 18 probands in whom mutations were detected, the same mutations were detected in between 1 and 8 first-degree relatives of 16 of the probands. All subjects with these mutations had baseline total cholesterol levels $\geq 6.8$ mmol/L, whereas relatives without the mutations had total cholesterol $\leq 6.8$ mmol/L. Two probands, L28 and L293, had only 1 first-degree relative available for testing and neither had the mutation. Their total cholesterol levels were 4.7 and 5.9 mmol/L, respectively. Only the mutation L405P in subject L293 has not been previously described and could not be confirmed in a relative. In 3

### Discussion

We identified 18 mutations among 29 heterozygous and 1 compound heterozygous Chinese FH patients, 11 of which were first found in this study (Table 2). They were scattered over the LDL receptor gene and changed conserved amino acid sequences in different functional domains of the mature protein. When family members were available for study, the mutations were shown to cosegregate with and therefore presumably responsible for the FH. In 18 probands in whom mutations were detected, the same mutations were detected in between 1 and 8 first-degree relatives of 16 of the probands. All subjects with these mutations had baseline total cholesterol levels $\geq 6.8$ mmol/L, whereas relatives without the mutations had total cholesterol $\leq 6.8$ mmol/L. Two probands, L28 and L293, had only 1 first-degree relative available for testing and neither had the mutation. Their total cholesterol levels were 4.7 and 5.9 mmol/L, respectively. Only the mutation L405P in subject L293 has not been previously described and could not be confirmed in a relative. In 3

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>TC</th>
<th>TG</th>
<th>HDL-C</th>
<th>LDL-C</th>
<th>Xanthomata</th>
<th>Corneal Arcus</th>
<th>CHD History</th>
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<td>M</td>
<td>43</td>
<td>16.0</td>
<td>1.30</td>
<td>1.08</td>
<td>14.3</td>
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<td>39</td>
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<td>Yes</td>
<td>No</td>
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<td>46</td>
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<td>1.34</td>
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<td>8.3</td>
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<td>7.9</td>
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<td>8.9</td>
<td>0.84</td>
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<td>M</td>
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<td>F</td>
<td>58</td>
<td>8.7</td>
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<td>1.62</td>
<td>1.28</td>
<td>7.2</td>
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<td>No</td>
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<tr>
<td>L79</td>
<td>F</td>
<td>19</td>
<td>10.9</td>
<td>1.39</td>
<td>1.04</td>
<td>9.6</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>L73</td>
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<td>38</td>
<td>9.1</td>
<td>0.98</td>
<td>1.02</td>
<td>7.6</td>
<td>Yes</td>
<td>No</td>
<td>Father</td>
</tr>
<tr>
<td>L24</td>
<td>M</td>
<td>44</td>
<td>8.2</td>
<td>1.15</td>
<td>0.79</td>
<td>6.9</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

**Clinical and Biochemical Features of the FH Patients**

**Patient** | **Sex** | **Age** | **TC** | **TG** | **HDL-C** | **LDL-C** | **Tendon Xanthomata** | **Corneal Arcus** | **CHD History** |
---|---|---|---|---|---|---|---|---|---|
L322 | M | 43 | 16.0 | 1.30 | 1.08 | 14.3 | Yes | No | Proband |
L243 | M | 11 | 11.0 | 1.09 | 0.99 | 9.5 | Yes | No | No |
L70 | F | 39 | 8.8 | 2.22 | 1.04 | 6.8 | No | Yes | No |
L246 | F | 46 | 10.1 | 1.34 | 1.23 | 8.3 | No | No | Father |
L166 | F | 31 | 7.4 | 0.96 | 1.24 | 5.7 | Yes | No | Mother |
L33 | M | 48 | 9.3 | 0.43 | 2.74 | 6.4 | Yes | No | No |
L139 | F | 44 | 9.7 | 0.82 | 1.49 | 7.8 | Yes | No | No |
L61 | M | 44 | 9.5 | 1.76 | 0.82 | 7.9 | No | Yes | No |
L66 | F | 26 | 8.9 | 0.84 | 1.20 | 7.3 | No | No | No |
L261 | M | 53 | 9.3 | 2.28 | 0.71 | 7.6 | No | Yes | Proband and brother |
L293 | F | 58 | 8.7 | 2.51 | 0.72 | 6.9 | No | Yes | Proband |
L28 | F | 55 | 12.0 | 1.63 | 1.04 | 10.2 | Yes | No | Proband |
L86 | M | 36 | 11.3 | 1.99 | 1.36 | 9.0 | Yes | No | No |
L253 | F | 54 | 8.8 | 3.64 | 1.22 | 5.9 | No | Yes | No |
L176 | M | 42 | 9.5 | 1.80 | 1.11 | 7.6 | No | Yes | No |
L265 | F | 80 | 11.3 | 0.93 | 1.03 | 9.8 | Yes | No | Son and daughter |
L39 | M | 47 | 10.3 | 1.37 | 1.02 | 8.7 | Yes | No | No |
L75 | F | 37 | 9.2 | 1.62 | 1.28 | 7.2 | Yes | No | No |
L79 | F | 19 | 10.9 | 1.39 | 1.04 | 9.6 | Yes | No | No |
L73 | M | 38 | 9.1 | 0.98 | 1.02 | 7.6 | Yes | No | Father |
L24 | M | 44 | 8.2 | 1.15 | 0.79 | 6.9 | Yes | No | No |

**FH** indicates familial hypercholesterolemia; **TC**, total cholesterol; **TG**, triglyceride; **HDL-C**, HDL cholesterol; **LDL-C**, LDL cholesterol; and **CHD**, coronary heart disease.

Concentrations are in mmol/L.

subjects, was obtained by electrophoresis in the presence of 10% glycerol at 2 different temperatures: room temperature and 4°C.

Among the 18 mutations detected in our FH patients, 11 were new mutations first found in this study: D69N in exon 3, I101F and G170X in exon 4, C308Y in exon 7, L393R and L405P in exon 9, 1706–1G $\rightarrow$T in intron 11, 1779delC in exon 12, A606V in exon 13, and C656F in exon 14 (Table 2). Three mutations, C308Y, I101F, and V408M, occurred in more than 1 unrelated patient.

Among these 30 FH patients, 6 previously reported polymorphisms were found (Table 3). The genotypic distributions were within Hardy-Weinberg equilibrium (data not shown).
subjects, L66, L253, and L39, no relatives were available to test. The mutation L393R in L66 was found in 2 other unrelated probands in this study (Table 2). The mutation G457R in L253 has been described before.2 Only the novel mutation 1779delC in subject L39 could not be confirmed in any other relative. Therefore, with the exception of mutations L405P and 1779delC, the functions of all mutations were supported either by family studies or previous reports. Although the functional effects of the mutations have not been confirmed by expression studies, they are unlikely to be artifacts.

There are now 29 mutations from 34 mutant alleles identified in the Chinese, including those from previous studies.2,11 In the present study there are 3 recurrent mutations C308Y and V408M in 2 and L393R in 3 unrelated patients (Table 2). The incidence rate, however, is too low to implicate common mutations due to a founder effect when compared with other populations, such as the Japanese.9 Screening of a large number of clinically diagnosed FH patients is necessary to affirm the prevalence and diagnostic value of these mutations.19

The G→A substitution at nucleotide 682 causes the mutation E207K. It was first described in a French Canadian population with a frequency of 2%.20 Such mutations had been detected in different ethnic groups and might have occurred independently during evolution.

A novel mutation was detected in intron 11 at 1 base position after the last base (position 1706) of exon 10. It was designated 1706–1G→T. Although it is probably a class 1 mutation,2 it remains to affirm whether it really creates mRNA splicing errors.

<table>
<thead>
<tr>
<th>TABLE 2. LDL-Receptor Mutations in Chinese FH Patients</th>
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</thead>
<tbody>
<tr>
<td>Patient Mutation</td>
</tr>
<tr>
<td>Mutations found in this study</td>
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<tr>
<td>L322 -44C→T</td>
</tr>
<tr>
<td>L664L</td>
</tr>
<tr>
<td>L243 D69N</td>
</tr>
<tr>
<td>L70 I101F</td>
</tr>
<tr>
<td>L246 G170X</td>
</tr>
<tr>
<td>L166 E207K</td>
</tr>
<tr>
<td>L33,139 C308Y</td>
</tr>
<tr>
<td>L61,66,261 L393R</td>
</tr>
<tr>
<td>L293 L405P</td>
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<td>L28,86 V408M</td>
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<td>L253 G457R</td>
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<td>L176 D471N</td>
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<tr>
<td>L265 1706-1G→T</td>
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<tr>
<td>L39 1779delC</td>
</tr>
<tr>
<td>L75 A606T</td>
</tr>
<tr>
<td>L79 A606V</td>
</tr>
<tr>
<td>L73 C656F</td>
</tr>
<tr>
<td>L24 V776M</td>
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</table>

Mutations not found in this study

- 18 Stop | G→A Promoter Class 1 | Reported11 |
- 637 del ACT | 637 del ACT Exon 4 Class 1 | Reported11 |
- Stop 207 | G→A Exon 4 Class 2/3 | Reported11 |
- C249Y | G→A Exon 5 Class 2/3 | Reported11 |
- Stop 462 | G→A Exon 10 Class 1 | Reported11 |
- L534P | T→G Exon 11 Class 5 | Reported11 |
- Del Exon 11-14 del | Reported11 |
- H562Y | L→T Exon 12 Class 5 | Reported11 |
- A606T | G→A Exon 13 Class 5 | Reported11 |
- Fs650 | 2015 del C Exon 14 Class 1 | Reported2 |
- Fs651 | 2017 del T Exon 14 Class 1 | Reported11 |
- Fs805 | 2477 del C Exon 17 Class 1 | Reported11 |

del indicates deletion; Fs, frameshift mutation. There were 18 mutations identified in our 21 Chinese FH patients. Novel mutations were those newly found in this study, and references are given for mutations that have been previously reported. *Mutation class was assigned according to the functional domains of mutations as described in references.
The C → T substitution at nucleotide 2054 in exon 14, resulting in the mutation P664L, was first reported in an Indian and occurs in different ethnic groups with frequencies ranging from 0.8% to 3.3% among FH patients. This mutation affects the intracellular processing of the LDL receptor protein and is possibly a common mutation worldwide.

The 2 mutations at the CpG dinucleotide in exon 13, A606V and A606T, due to a C-to-T change at nucleotide 1880 and a G-to-A change at nucleotide 1879, respectively, are the only mutations identified in exon 13, and they have been observed only in the Chinese. In contrast, mutations in exons 9 to 12, which code for equivalent functional parts of the LDL receptor as does exon 13, are frequently detected in different populations. The A606T mutation has been found in a Chinese subject in mainland China having milder hypercholesterolemia than our subject, suggesting an environmental effect on phenotypic expression. However, such diversified phenotypic expressions of A606T could also be the result of a multigenic trait.

The allelic frequencies of the polymorphisms found in our FH patients (Table 3) were different from those of the whites and similar to those of the Japanese. The χ² analysis showed that the allelic frequency of the AvaII polymorphism at exon 13 (0.880.12) was significantly different (χ² = 19.2, P < 0.001) from that reported for whites (0.51/0.49), but there was no significant difference (χ² = 0.36, NS) compared with Japanese subjects (0.80/0.20). Comparison analysis with the AciI, HincII, and MspI polymorphisms in exons 11, 12, and 15, respectively, gave similar results. The number of subjects (30) in our study might be slightly small for such statistical calculation, but the difference was obvious and indicated an isolation between white and Oriental populations. A large screening study is required to test whether such isolation really exists.

The 9 patients in whom we did not detect any LDL-receptor mutation constituted 30% of all our FH patients. This percentage was consistent with a previous study on 10 Chinese FH patients and higher than in another study on 20 Danish FH patients, in which the corresponding percentages were 30% and 10%, respectively. It is possible that mutations were not in the known coding regions or

### Table 3. Polymorphisms Found in the LDL-Receptor Gene Among the 30 Chinese FH Patients

<table>
<thead>
<tr>
<th>Exon</th>
<th>Nucleotide Change</th>
<th>Restriction Site</th>
<th>Frequency of the Rare Allele</th>
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</thead>
<tbody>
<tr>
<td>2</td>
<td>81C→T</td>
<td>SfaNI</td>
<td>0.03 (2/60)</td>
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<tr>
<td>10</td>
<td>1413G→A</td>
<td>BsmAI</td>
<td>0.33 (20/60)</td>
</tr>
<tr>
<td>11</td>
<td>1617C→T</td>
<td>AciI</td>
<td>0.22 (13/60)</td>
</tr>
<tr>
<td>12</td>
<td>1773C→T</td>
<td>HincII</td>
<td>0.13 (8/60)</td>
</tr>
<tr>
<td>13</td>
<td>1959T→C</td>
<td>AvaII</td>
<td>0.12 (7/60)</td>
</tr>
<tr>
<td>15</td>
<td>2232G→A</td>
<td>MspI</td>
<td>0.03 (2/60)</td>
</tr>
</tbody>
</table>

All polymorphisms have been previously reported.²¹⁻²⁶
promoter of the LDL receptor gene. Meanwhile, some patients may have a defective apolipoprotein B, with mutations other than the G10708→A substitution that leads to the R3500Q mutation, such as the R3571C or other unidentified mutations. Defective apolipoprotein B could be examined by exclusion mapping with flanking microsatellite markers.

In summary, LDL receptor gene mutations in the Chinese are largely different from those in other populations. No common mutation of diagnostic value for FH has been established. Mutation analysis in the LDL receptor gene of a large number of Chinese heterozygous FH patients should be continued, and possible functional defects in the LDL receptor caused by the newly found mutations should also be sought. This study population is too small to draw any conclusion about genetic epidemiological features of the Chinese as a whole, and screening for LDL receptor mutations in a large number of clinically diagnosed FH patients is required.

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References

18. Deleted in proof.
Mutations in the Low-Density Lipoprotein Receptor Gene in Chinese Familial Hypercholesterolemia Patients

Ying-Tat Mak, Chi-Pui Pang, Brian Tomlinson, Jun Zhang, Yuen-Shan Chan, Tony W. L. Mak and John R. L. Masarei

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