Identification of Recurrent and Novel Mutations in Exon 4 of the LDL Receptor Gene in Patients With Familial Hypercholesterolemia in the United Kingdom

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A group of 200 patients with familial hypercholesterolemia (FH) who were attending lipid clinics in the London area have been screened for four known point mutations and a microdeletion in exon 4 of the low density lipoprotein receptor gene by polymerase chain reaction (PCR) amplification of genomic DNA and either enzyme digestion of the product or hybridization with allele-specific oligonucleotides. A point mutation of Ser^195—Leu that was initially described in a Puerto Rican family was found in one patient of Polish origin on a different haplotype from that described originally and thus is likely to have occurred independently. A 3-bp deletion that causes deletion of amino acid Gly^207 was found in six of the patients, who were all of Jewish origin and who shared the same haplotype for the mutant allele. A mutation of Asp^206—Glu that has been described in the Afrikaner population was found in three patients, two of UK origin and one a recent immigrant from South Africa. In all cases the haplotype of the mutant allele was compatible with that described in the original patient. The mutations at Asp^206 reported in South African patients and at Gly^207 reported in French Canadian patients were not detected in this sample. However, two additional mutations have been identified in this sample: the first, a 2-bp deletion in codon 206 that was found in five patients, all of British ancestry, and the second, a point mutation in a single patient of Irish origin that creates a stop codon at residue Cys^331p. Of the 200 FH patients in the sample, the molecular defect in 16 (8%) could be detected by PCR using three different tests. Thus, based on the results from analysis of exon 4, the data suggest that during screening of FH patients who have been selected from a population of heterogeneous origin, only a limited spectrum of reported mutations will be found and the occurrence of the same mutation in different patients does not necessarily imply that they share a common ancestor, especially for those mutations occurring at 5'-CpG-3' dinucleotides. (Arteriosclerosis and Thrombosis 1993;13:56-63)

KEY WORDS • LDL receptor gene • familial hypercholesterolemia • mutations

Familial hypercholesterolemia (FH) is a mono-genic disorder that is inherited as an autosomal dominant disease. In the classical clinical descriptions of the disorder more than 50 years ago, it was shown to be associated with a twofold to threefold elevation of serum cholesterol together with tendinous xanthomas, arcus cornea, and premature atherosclerosis in many of the affected heterozygotes. The frequency of the disease in most communities is one in 500, making FH one of the most common monogenic diseases known. In some individuals with the clinical diagnosis of FH, the disorder is caused by a mutation in the apolipoprotein B (apoB) gene, but in the majority of patients, the disorder is caused by different mutations in the low density lipoprotein (LDL) receptor. Because of founder effects, the frequency of the disease and of particular mutations is much higher in some countries with populations that have been isolated by cultural or geographic boundaries. For example, in French Canadians the same deletion is found in 60% of FH patients, and in the Christian Lebanese one mutation is responsible for the disorder in 98% of all FH patients, while in Afrikaners in South Africa three mutations are responsible in more than 95% of FH patients, with the most common mutation being found in two thirds of all patients.

A number of mutations in the LDL receptor gene in FH patients have now been described. Of these mutations, more than 30 are deletions, and, where characterized in detail, many of these are thought to be caused by recombination between Alu sequences. The observed frequency of deletions in studies of FH patients who have been screened has been between 2% and 6%. In addition, more than 10 single-base-pair (bp) changes have been reported, but overall the known mutations account for a very small number of all
patients, leaving the majority of defects responsible for FH uncharacterized. Thus, in a genetically heterogeneous population, the number of different mutations is likely to be high, even though each particular mutation may be common to several families. The frequency of any particular mutation will be largely dependent on its time of occurrence in a given population and on fluctuations in population size and migration patterns.

In recent years we have collected samples from more than 200 FH patients who were attending lipid clinics in London and the south of England in order to investigate the spectrum of mutations in the LDL receptor gene and to determine their approximate frequencies as the initial step in identifying and characterizing all patients with FH in this area of the United Kingdom. All of the patients have FH as defined by classical clinical and biochemical criteria. This paper describes the results of screening the 200 patients for five of the reported mutations in exon 4 of the LDL receptor gene. Four of the 1-bp substitutions that cause FH have been described in this exon are a G—A that changes Asp154 to Asn,14 a G—T that changes Ser158 to Leu,15 a C—G that changes Asp196 to Glu,8 and a G—A that changes Glu207 to Lys,16 the three last occurring at a 5'-CpG-3' (CpG) dinucleotide. In addition, a 3-bp deletion in exon 4 has been described that causes the deletion of amino acid Gly157.10,17

Methods

Patients

Complete details of the patient selection have been described elsewhere.13 In brief, the patient sample consisted of 189 heterozygous and 11 homozygous patients (all apparently unrelated) with the diagnosis of FH who were attending lipid clinics in the London area. Previously defined standard diagnostic criteria for FH were applied, including a total serum cholesterol level of more than 7.5 mmol/l and plasma level of LDL cholesterol above 4.9 mmol/l, with tendon xanthomas and/or premature coronary artery disease in the patient or a first-degree relative. Of the 200, 35% have tendon xanthomas, and in the families of 44 patients, the involvement of a mutation in the LDL receptor gene has been confirmed by cosegregation of restriction fragment length polymorphisms (RFLPs) of the gene with hypercholesterolemia. Patients with the mutation in the gene for apoB causing familial defective apoB were excluded.5

Amplification of Genomic DNA

Exon 4 of the LDL receptor gene was amplified by polymerase chain reaction (PCR)18 with two sets of oligonucleotide primers: primer 1, 5' CATCCATCCTCCTGCAGCCC3' from the intron 3—exon 4 boundary, and primer 2, 5' GCACCATACCGGATTTTCCC3' from the exon 4—intron 4 boundary, together amplifying a 405-bp fragment designated as fragment I; and primer 3, from bp 528 to bp 547 in exon 4 (5' CGACTGCGAA- GATGGCTCGGAG3') and primer 4 in intron 4 (5' GG-GACCCAGGGACAGTGATAGCA3'), giving a 242-bp fragment designated as fragment II. Oligonucleotides were obtained from Severn Biotech Ltd., Kidderminster, UK. The amplifications were performed in an automated thermal cycler (Cambio, Cambridge, UK) with Thermus aquaticus (Tag) DNA polymerase (Perkin Elmer-Cetus, Norwalk, Conn.) in the buffer as recommended by the manufacturer and a total volume of 50 μl. The conditions were 95°C for 5 minutes and 68°C for 6 minutes once and subsequently at 95°C for 1 minute and 68°C for 6 minutes for 30 cycles.

Identification of Mutations

To detect the Asp154—Asn mutation, PCR fragment I (20 μl) was digested with 10 units Mbo II (Anglian Biotech Ltd.) for 16 hours in a total volume of 30 μl. The digestion mixture was then analyzed by electrophoresis through a 1.2% agarose gel (Ultrapure Agarose, BRL). To detect the Asp206—Glu mutation, PCR product II (20 μl) was digested with 12 units Dde I (Anglian Biotech Ltd.) for 16 hours in a volume of 30 μl in the supplied buffer. The digested fragments were then analyzed by electrophoresis on a 7.5% polyacrylamide gel. DNA bands were visualized by UV transillumination of ethidium bromide-stained gels.

Blotting and Hybridization With Allele-Specific Oligonucleotides (ASOs)

To detect the Ser158—Leu and the Glu207—Lys mutations, PCR fragment II was fractionated by electrophoresis for 3 hours on a 1% agarose gel, which was then denatured in 1.5 M NaCl/0.5 M NaOH for 30 minutes at room temperature and then "double blotted" (10 μl) between two pieces of nylon membrane (Hybond-N+, Amersham, UK), using the denaturing solution as the transfer buffer. The DNA was bound to the filters by UV light from a transilluminator for 1 minute. Three pairs of ASOs were used: for the codon 156 mutation, ASO 1 (5' AAGATGCTTGGATGAGT3') and its normal allele ASO 2 (5' AAGATGCTCGGATGAGT3'); for the codon 207 mutation, ASO 3 (5' CAAATCTGACGAGAAACT3') and its normal allele ASO 4 (5' CAAATCTGACGAGAAACT3'); and for the codon 197 deletion, ASO 5 (5' TGTGATGCCC3') and ASO 6 (5' GTGATGATGCCC3'), the respective normal alleles. Positive controls for the mutations were synthesized as described previously by PCR amplification with oligonucleotide primers that introduced the specific mutations.19 All ASOs were labeled at the 5' end with T4 polynucleotide kinase (BRL, Paisley, UK) and adenosine 5'-[α-32P]triphosphate (Amersham, UK) to a specific activity of approximately 0.1 μCi/pmol. The filters were hybridized for 1 hour in 5× saline—sodium phosphate—EDTA buffer (SSPE) 1× SSPE is 0.9% NaCl, 50 mM sodium phosphate, and 5 mM EDTA)/0.5% sodium dodecyl sulfate (SDS)/5× Denhardt's solution at 42°C for ASOs 1, 2, 3, and 4 and at 33°C for ASOs 5 and 6. The filters were then washed for 3 minutes at room temperature in 2× SSPE and 0.2% SDS for all ASOs and subsequently for 10 minutes in 0.2× SSPE and 0.1% SDS at 42°C for ASOs 1, 2, 3, 4 and at 39°C for ASOs 5 and 6. An additional 10-minute wash in 0.1× SSPE and 0.1% SDS was done for ASOs 1, 2, 3, and 4. Exposure time for autoradiography was 2—16 hours.

Haplotype Analysis

Genotypes for six LDL receptor gene RFLPs were determined either by restriction digestion of genomic DNA followed by Southern blotting as described for
FIGURE 1. Mutations in exon 4 of the low density lipoprotein receptor (LDL-R) gene. Diagram of the 3' end of exon 4 of the LDL-R gene and its flanking intron shows the positions of four previously described mutations\(^7\) and the two new mutations described in this paper. Deleted bases are indicated by hatched boxes over the sequences, and single-base changes are indicated by vertical arrows (T) together with the identity of the codon(s) concerned. Oligonucleotide primers used for amplification are shown by horizontal arrows (5'-3'). Occurrence of mutations in the sample of patients from the United Kingdom is summarized in Table 1.

Results

A total of 211 LDL receptor-defective alleles (11 homozygous and 189 heterozygous patients) were screened for the known mutations in exon 4 of the LDL receptor gene shown in Figure 1, and the results are summarized in Table 1. All patients in whom a mutation was found were heterozygous for FH. In this sample, no patients with the Asp\(^{154}\)→Asn or the Glu\(^{206}\)→Lys mutation were found. In one patient the Ser\(^{156}\)→Leu mutation was detected (Figure 2), and the Asp\(^{206}\)→Glu mutation was found in three (Figure 3). The sequence of both of these mutations was confirmed by DNA sequencing of the amplified fragments (Figures 4a and 4b). In one patient a different abnormal restriction fragment pattern was observed when fragment II was digested with \(\text{Dde I}\), during which the 134-bp fragment was cut into 80- and 54-bp fragments. Sequencing amplified fragment II from this patient revealed a single base substitution (C→A) that changed codon Cys\(^{210}\) to stop (Figure 4b). In addition, extra bands were observed in 11 of the patients after polyacrylamide gel electrophoresis of \(\text{Dde I}\)-digested fragment II. These bands migrated more slowly than the normal bands and behaved like heteroduplexes, which are characteristic of insertions or deletions of a few bases.\(^{25}\) These extra bands were observed in two different patterns, one of which was found in six patients and the other in five patients (Figure 3). Hybridization of the PCR fragments with ASOs strongly suggested that one of these abnormal patterns, i.e., one observed in six patients, was due to the presence of the 3-bp deletion of the codon for Gly\(^{197}\) in one allele (Figure 2). This was confirmed by direct sequencing of the PCR product (Figure 4c).

The genotypes of patients with detected mutations were determined at six polymorphic sites within the LDL receptor gene. In instances where a patient or a relative was homozygous for the RFLP, the haplotype of the defective allele could be defined unambiguously. In instances where the patient was heterozygous for the
**TABLE 1. Frequency of Mutations in Exon 4 of the LDL Receptor Gene**

<table>
<thead>
<tr>
<th>Mutation</th>
<th>No. in this study*</th>
<th>Ethnic origin of patients</th>
<th>Haplotype of the defective LDL receptor gene†</th>
<th>Previous description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asp154→Asn</td>
<td>0</td>
<td></td>
<td></td>
<td>14</td>
</tr>
<tr>
<td>Ser156→Leu</td>
<td>1</td>
<td>Poland (1)</td>
<td>− + − + − +</td>
<td>15</td>
</tr>
<tr>
<td>Asp206→Glu</td>
<td>3</td>
<td>Afrikaner (1)</td>
<td>− + − − − −</td>
<td>8</td>
</tr>
<tr>
<td>Glu207→Lys</td>
<td>0</td>
<td>British (2)</td>
<td></td>
<td>16</td>
</tr>
<tr>
<td>3-bp deletion (Gly197)</td>
<td>6</td>
<td>Jewish (6)</td>
<td>− + − + − −</td>
<td>17</td>
</tr>
<tr>
<td>Cys210→Stop</td>
<td>1</td>
<td>British (1)</td>
<td>− + +/− X</td>
<td>None</td>
</tr>
<tr>
<td>2-bp deletion (bases 694 and 695)</td>
<td>5</td>
<td>British (5)</td>
<td>− + + − +</td>
<td>None</td>
</tr>
</tbody>
</table>

*Number found in the group of 211 low density lipoprotein receptor (LDL-R) defective alleles.
†Genotype as determined by haplotype; “+” or “−” indicates the presence or absence, respectively, of the cutting site. +/-, Unable to determine unambiguously; T, Taq I; S, Stu I; H, Hinc II; A, Ava II; V, Pvu II; N, Nco I; X, not determined.
‡Additional polymorphisms in the haplotype for this mutation are Sph I- and Apa L1-5’-

RFLP, the haplotype was deduced by using information from available relatives and assuming that no recombination had occurred. An example of this is presented in Figure 5 for the Asp206→Glu mutation. As shown in Table 1, the other two patients with this mutation have a haplotype compatible with that observed in the family, although it cannot be determined unambiguously. For the Gly197 deletion, all six patients have the same (or compatible) haplotype for the defective allele, which differs from the haplotype that is deduced as common for all 2-bp-deletion patients (Table 1).

The biochemical and clinical characteristics of the patients in whom mutations in exon 4 were detected are presented in Table 2. The untreated total plasma cholesterol and LDL cholesterol levels varied widely, even within groups of patients with the same mutation.

**Discussion**

The general population in London is very mixed with regard to racial and ethnic origin, and therefore, FH patients will also vary widely in their geographic background and exhibit the spectrum of mutations found elsewhere in the world. As part of a systematic approach to identify mutations that cause FH in the United Kingdom, this paper describes the results of screening for known mutations in exon 4 of the LDL receptor gene in 211 alleles in patients with a clinical diagnosis of FH who were attending lipid clinics in the London area. In this sample we have previously identified nine patients with gross deletions of the LDL receptor gene. Of the five previously reported mutations in this exon, three were represented in the London sample. No patients were found with the Asp154→Asn mutation.

**Figure 2.** Detection of mutations in exon 4 by hybridization with allele-specific oligonucleotides (ASOs). Amplified fragment I (see text) was fractionated by electrophoresis on an agarose gel and transferred to nylon membranes (blots) as described in “Methods.” Panel a: Detection of Ser156→Leu mutation. Blot A was hybridized to a normal-sequence ASO and blot B to the ASO specific for the mutated sequence. Lane 10 is a positive control whose size is different from that of the exon 4 polymerase chain reaction product. DNA fragments in lanes 1, 2, 3, 5, 7, 8, and 9 are negative for the Ser156→Leu mutation. Sample in lane 4 is positive for that mutation. Panel b: Detection of the Gly197 deletion alleles. Blot A was hybridized to an ASO specific for the normal sequence. Blot B was hybridized to an ASO containing the deleted sequence. Lanes 3, 4, and 5 are positive for that mutation, and lanes 1 and 2 are negative.

**Figure 3.** Detection of mutations in exon 4 by polyacrylamide gel electrophoresis. Fractionation of polymerase chain reaction fragment II by polyacrylamide gel electrophoresis after digestion with Dde I and staining with ethidium bromide as described in “Methods.” Each lane contains Dde I-digested amplified DNA from patients heterozygous for the following mutations: Cys210→Stop (lane 1), 2-bp deletion (lane 2), Gly197 deletion (lane 3), and Asp206→Glu (lane 4); lanes 5 and 6 show the normal restriction fragment pattern.
reported in Afrikaners in South Africa or the Glu207—Lys found in a French Canadian and a Mexican patient. However, the other two previously described point mutations were found in the London sample. The Ser156—Leu change that has been described in a Puerto Rican family living in the United States was identified in one patient from London, who is a second-generation immigrant from Poland. The haplotype of the Leu allele in this patient differs in the NcoI and AvaII polymorphisms from the haplotype reported in the initial description of this mutation, and thus, it is likely to have arisen independently. A previously unreported 1-bp change was also found in one patient. This was a C—>A transversion at a CpG dinucleotide that created a termination codon at amino acid Cys109. This truncated protein is likely to be degraded intracellularly, and experiments are under way to investigate this possibility.

The Asp206—Glu mutation, which occurs in 65% of patients with FH in the Afrikaner population in South Africa, was found in three patients in this sample. One of the patients identified in the London sample is from South Africa but is not of Afrikaner origin, while the other two have lived in England for many generations with no evidence of recent migration. For all three patients the genotypes were consistent with the Glu mutation being carried on the same six-polymorphism haplotype (determined unequivocally in one family), which was identical with the haplotype for this mutation reported in the Afrikaner population. This mutation has also been reported in an FH patient of English ancestry in North America, in whom the mutation is on a haplotype differing from that in the South African patient only at the 3′Apa I polymorphism, which is downstream of the LDL receptor gene. The majority of the Afrikaner population is of Dutch origin, but the
Asp<sub>206</sub>→Glu mutation has not been found in FH patients in The Netherlands despite an extensive search (J. Kastelein, personal communication). However, an estimated 5% of the Afrikaans-speaking population in 1867 were of English descent, and therefore, it is possible that this mutation occurred originally in an individual in England.

Of the point mutations that have been described in the LDL receptor gene, almost half have occurred at a CpG dinucleotide "hot spot." Indeed, of the described 1-bp substitutions that cause human disease, the CpG dinucleotide is involved most frequently, accounting for about 35% of reported mutations, and there is evidence that many of them have occurred independently.

### Table 2. Clinical Characteristics of Individuals With Various Mutations in Exon 4 of the LDL Receptor Gene

<table>
<thead>
<tr>
<th>Mutation</th>
<th>FH number</th>
<th>Sex</th>
<th>Age (years)</th>
<th>TC (mmol/l)</th>
<th>TG (mmol/l)</th>
<th>HDL-C (mmol/l)</th>
<th>LDL-C (mmol/l)</th>
<th>TX</th>
<th>CAD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ser&lt;sub&gt;156&lt;/sub&gt;→Leu</td>
<td>FH 181</td>
<td>M</td>
<td>47</td>
<td>11.30</td>
<td>1.99</td>
<td>1.25</td>
<td>9.15</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>Asp&lt;sub&gt;206&lt;/sub&gt;→Glu</td>
<td>FH 104</td>
<td>F</td>
<td>29</td>
<td>7.90</td>
<td>0.90</td>
<td>1.24</td>
<td>6.25</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>Cys&lt;sub&gt;210&lt;/sub&gt;→stop</td>
<td>FH 105</td>
<td>M</td>
<td>50</td>
<td>11.20</td>
<td>0.90</td>
<td>0.83</td>
<td>9.96</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>3-bp deletion (Gly&lt;sub&gt;190&lt;/sub&gt;)</td>
<td>FH 154</td>
<td>F</td>
<td>61</td>
<td>13.30</td>
<td>1.70</td>
<td>1.70</td>
<td>10.83</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>FH 40</td>
<td>F</td>
<td>43</td>
<td>8.90</td>
<td>1.13</td>
<td>1.45</td>
<td>6.93</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>FH 13</td>
<td>F</td>
<td>39</td>
<td>11.00</td>
<td>0.80</td>
<td>0.96</td>
<td>9.67</td>
<td>Y</td>
<td>N</td>
<td></td>
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<tr>
<td>FH 21</td>
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<td>52</td>
<td>10.70</td>
<td>1.50</td>
<td>1.29</td>
<td>8.62</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
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<tr>
<td>FH 41</td>
<td>F</td>
<td>58</td>
<td>14.00</td>
<td>2.20</td>
<td>1.10</td>
<td>12.60</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
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<tr>
<td>FH 46</td>
<td>M</td>
<td>36</td>
<td>10.40</td>
<td>0.73</td>
<td>1.24</td>
<td>8.80</td>
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<td>N</td>
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<tr>
<td>FH 47</td>
<td>M</td>
<td>49</td>
<td>12.40</td>
<td>1.45</td>
<td>1.14</td>
<td>10.60</td>
<td>Y</td>
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<tr>
<td>FH 118</td>
<td>M</td>
<td>36</td>
<td>9.30</td>
<td>0.77</td>
<td>1.11</td>
<td>6.85</td>
<td>Y</td>
<td>N</td>
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<tr>
<td>2-bp deletion of bases 694 and 695</td>
<td>FH 9</td>
<td>M</td>
<td>40</td>
<td>8.90</td>
<td>0.69</td>
<td>0.98</td>
<td>6.60</td>
<td>Y</td>
<td>N</td>
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<tr>
<td>FH 36</td>
<td>M</td>
<td>49</td>
<td>10.60</td>
<td>1.10</td>
<td>0.96</td>
<td>9.10</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
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<tr>
<td>FH 69</td>
<td>M</td>
<td>43</td>
<td>12.80</td>
<td>1.15</td>
<td>1.48</td>
<td>10.79</td>
<td>Y</td>
<td>N</td>
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<tr>
<td>FH 53</td>
<td>M</td>
<td>46</td>
<td>9.60</td>
<td>1.20</td>
<td>1.10</td>
<td>7.95</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
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<tr>
<td>FH 186</td>
<td>F</td>
<td>34</td>
<td>9.70</td>
<td>0.90</td>
<td>1.40</td>
<td>7.77</td>
<td>Y</td>
<td>N</td>
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</tbody>
</table>

**LDL,** low density lipoprotein; **FH,** familial hypercholesterolemia; **TC,** total cholesterol; **TG,** triglycerides; **HDL-C,** high density lipoprotein cholesterol; **LDL-C,** low density lipoprotein cholesterol; **TX,** tendinous xanthomas; **CAD,** coronary artery disease.

*The number is an arbitrary identification number assigned to the patient for this study.
†Age at the time of cholesterol measurement.
‡Untreated levels.
§Calculated by Friedewald's formula: LDL-C=TC−HDL-C−TG/2.2 mmol/l.
||Positive diagnosis of CAD as myocardial infarct, coronary artery bypass graft, or angina pectoris diagnosed by coronary angiography or positive exercise test.
at the same site in different individuals. There have been similar examples in the LDL receptor gene, and Leitersdorf et al have reported that a mutation at a CpG in codon Glu249 has occurred on a gene with a different haplotype in a French Canadian patient and in a Mexican FH homozygote. We have recently shown that a mutation at a CpG dinucleotide in the codon for Pro664 in exon 14 of the LDL receptor gene has occurred independently at least twice and probably three times according to haplotype analysis. Although recombination between chromosomes during meiosis could, over long periods of time, result in such a mutation being "spread" onto several haplotypes, analysis with multiple variable sites that flank the mutation suggests that this explanation is unlikely. CpG dinucleotides are hot spots for mutations through a mechanism that involves deamination of methylated cytosine to thymidine. Transversions are also known to occur at CpG dinucleotides at higher frequencies than expected. Of the five known point mutations in exon 4 of the LDL receptor gene, four occur at a CpG dinucleotide, of which two are transitions and two are transversions. Two of the mutations have occurred at the same CpG dinucleotide: the Asp747→Glu, which is a transversion, and the Glu249→Lys, which is a transition. There are 26 CpG dinucleotides in this 384-bp exon, which is approximately one in 15 bases, compared with one in 22 bases on average in the remainder of the coding region of the LDL receptor gene.

The other mutations found in this exon are two small deletions, both of which are relatively common in our sample. The Gly597 deletion occurs in six of the FH patients in London, who are all of Jewish origin and one of whom is a recent immigrant from South Africa. All alleles carrying the deletion have or are consistent with the same six RFLP haplotypes. This mutation was originally described in an FH homozygous patient living in the United States, and it causes a reduced rate of intracellular processing of the receptor protein, leading to a reduced number of receptors on the cell surface. This deletion has recently been identified at a high frequency in patients of Lithuanian Jewish origin, with the reported haplotype of the chromosome carrying the deletion being compatible with that observed in the patients from London. The other small deletion is the next most frequent mutation in our sample, occurring in five of the patients, all of which are consistent with the mutations being on the same haplotype. All of these patients are of English descent. If the RNA is spliced normally, the deletion would lead to a frameshift and stop codon and create a truncated protein of the first 205 residues of the LDL receptor with an additional 11 abnormal residues. By comparison with other mutant forms of receptor protein, it is likely that this protein would be rapidly degraded in the cell. Taken together, these two deletions were found in 11 of 211 alleles, accounting for approximately 5% of all FH patients in the London sample, although because of differences in ethnic makeup it is likely that the frequency of these deletions will vary in other regions of the United Kingdom.

The finding of two small deletions within such a small region of the LDL receptor gene is noteworthy. Multiple mechanisms have been suggested to be responsible for deletions of fewer than 20 bp. In 93% of the genes with such deletions the DNA sequence involved contained direct repeats of between 2 and 8 bp that either included or partially overlapped the deleted bases. In the case of the 3-bp deletion in the LDL receptor gene, there is a repeat sequence of TGG-TGG, with the internal GGT being deleted. The 2-bp deletion has a dinucleotide repeat GA-C-GA overlapping the AC deletion. In either case the exact mechanism of the deletion itself is not obvious. The 2-bp deletion is contained within a hexanucleotide with homology to a reported hot spot for deletions (consensus TGAGGA). This sequence has similarity to DNA polymerase-α "arrest" sequences, suggesting that arrest of synthesis may be involved in the mechanism of the deletion. Currently, exon 4 of the LDL receptor gene has eight different reported mutations that cause FH, and because of the sequence, the high CpG content, and the fact that exon 4 codes for a critical region in the binding domain of the LDL receptor, it is likely that others remain to be found.

In each case where several patients with the same mutation were identified, we found a wide range of biochemical and clinical expression of the mutant alleles. Because of the small number of individuals involved, it is not possible at present to draw any conclusions about the relation between a specific mutation and the biochemical and clinical parameters, but this will become possible as more patients with defined mutations in the LDL receptor gene become available.

Our study confirms the large number of different mutations of the LDL receptor gene present in an urban population of mixed origin. In the sample of 200 patients, large deletions or rearrangements of the LDL receptor gene were detected in nine individuals, while five individuals with the Pro664→Leu mutation and five with the Glu249→Lys mutation have previously been identified. We have now detected specific mutations in exon 4 in an additional 16 patients. Since the apoB Arg500→Gln mutation has been detected in 3% of FH patients in London, in total the molecular defect has been identified in more than 20% of these patients by using techniques that could be routinely applied in any laboratory.

Acknowledgments

We thank Dr. G.R. Thompson and Dr. A. Munro for access to patients, Sue McCarthy for help with sample collecting, the patients and their relatives for their cooperation, and Gina Deeley for help in preparing the manuscript.

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doi: 10.1161/01.ATV.13.1.56
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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