Characterization of Deletions in the LDL Receptor Gene in Patients With Familial Hypercholesterolemia in the United Kingdom

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A sample of 200 patients with a clinical diagnosis of heterozygous (189) or homozygous (11) familial hypercholesterolemia (FH) attending lipid clinics in the London area have been screened for the presence of major gene defects in the low density lipoprotein (LDL) receptor gene by Southern blotting of genomic DNA with specific probes. This study is part of a project to determine the frequency of known mutations in the LDL receptor gene in this population. A new polymorphism for the enzyme Bgl II was identified by hybridization with a probe specific for the promoter plus exon 1 of the LDL receptor gene. The observed frequency of the rare allele, characterized by a Bgl II fragment of 13 kb compared with 10 kb for the common allele, was 0.08 in this group of FH patients. Several individuals who were heterozygous for the rare allele were also heterozygous for a mutation elsewhere in the LDL receptor gene that is known to cause FH. Eight different mutations, seven deletions and one duplication, were detected in a total of nine patients, accounting for 4.5% of the mutant alleles in this group. Three of the mutations are apparently identical to deletions that have been described previously in FH patients of British or European origin, while the remaining five have not been described. Two of these were in patients of Polish and Asian Indian origin, while the other three were in patients of apparently British ancestry. (Arteriosclerosis and Thrombosis 1992;12:762–770)

KEY WORDS • atherosclerosis • polymorphisms • mutations • genetic screening • hyperlipidemia

Familial hypercholesterolemia (FH) is an inherited disease caused by mutations in the gene for the low density lipoprotein (LDL) receptor that affects approximately one person in every 500 in most populations, making it one of the more common inherited diseases of metabolism.

Individuals in whom one LDL receptor gene is defective have a plasma cholesterol concentration approximately twice that of the mean of the whole population, and as a result, excessive amounts of cholesterol are deposited in extrahepatic tissues. This leads to the formation of xanthomas, characteristically in tendons, and to accelerated atherosclerosis, with the result that heterozygous FH patients frequently suffer from premature coronary heart disease. Homozygous individuals, with two defective genes, are more severely affected and rarely reach the age of 30 unless stringent methods to reduce plasma cholesterol are applied.

Homozygous FH is rare and clinically unmistakable, but it is often not possible to make an accurate diagnosis of heterozygous FH on the basis of clinical criteria alone; many hypercholesterolemic patients can only be described as "probable FH" or even "possible FH" unless they have tendon xanthomas or a well-defined family history of hypercholesterolemia and premature coronary heart disease.

For this reason a diagnostic test based on the identification of the specific mutation in the LDL receptor gene would prove invaluable, permitting an accurate diagnosis on which treatment and counseling could be based. However, the marked heterogeneity of FH at the level of the gene precludes the development of any simple screening test at present, and the mutation in each patient has to be determined de novo. It has recently been estimated that there are at least 180 different defective alleles of the LDL receptor gene in known homozygous FH patients from many different parts of the world, only a few of which have been characterized in terms of the underlying mutation.

On the other hand, in some isolated populations a founder effect has resulted in a higher-than-normal frequency of FH patients in whom the majority have the same or one of a small number of mutant alleles. Examples of these include the Lebanese, French Canadians, Afrikaners in South Africa, and Jewish individuals of Lithuanian origin. Although it is unlikely that a predominant founder gene effect will be observed in more heterogeneous populations of FH patients, such as that in the United Kingdom, it is possible that a small number of mutations may be relatively common, particularly in groups of similar cultural or ethnic background. How-

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ever, no systematic survey of the frequency of different mutations in the LDL receptor gene in the FH population in the United Kingdom has been undertaken. Therefore, we have selected a group of 200 patients with a clinical diagnosis of FH who reside in the southeast of England and in whom we intend to determine the frequency of the previously described mutations in the LDL receptor gene. In this article we describe the identification of those patients in the group in whom the mutant allele of the LDL receptor gene has a major deletion or rearrangement that can be detected by Southern blotting of genomic DNA with specific probes.

**Methods**

**Selection of Patients**

The majority of the patients in this study (189 of 200) were selected at random from a group of patients with a clinical diagnosis of heterozygous FH who have been attending one of three lipid clinics in the London area during the past few years (Hammersmith Hospital, Charing Cross Hospital, and St. Mary's Hospital). The diagnostic criteria for definite heterozygous FH in these clinics require that the patient has a plasma cholesterol concentration of 7.5 mmol/l or above (6.7 mmol/l if 16 years old or younger) and that detectable tendon xanthomas are present in the patient or a first- or second-degree relative. If no tendon xanthomas are present, the patient is given a diagnosis of possible FH if a hypercholesterolemic first-degree relative suffered a myocardial infarction before the age of 60 years (or 50 years in a second-degree relative). The other 11 patients had homozygous FH, based on a plasma cholesterol level of >15 mmol/l, the presence of cutaneous and tendon xanthomas, cardiovascular involvement before puberty, and hypercholesterolemia in both parents. Clinical details of most of these homozygous individuals have been described previously. Genotype analysis based on four polymorphic sites in the LDL receptor gene showed that at least seven of these clinically homozygous patients were compound heterozygotes with two different mutant alleles (J. Webb and A.K. Soutar, unpublished observations). Thus, the sample of patients comprised not less than 207 defective alleles for the LDL receptor gene.

All but one of any known related individuals were excluded from the study, but no attempt was made to select patients on the basis of cultural and ethnic origin. Patients with a clinical diagnosis of FH who were known to carry the gene for familial defective apolipoprotein B_{100} were excluded from this study.

**Southern Blotting**

Genomic DNA was isolated from frozen whole blood (10 ml) or frozen packed blood cells by lysis of the cells with Triton X-100 followed by phenol/chloroform extraction, essentially as described previously. The integrity and approximate concentration of the DNA were determined by electrophoresis on 0.7% (wt/vol) agarose gels stained with ethidium bromide; a known amount of lambda phage DNA cut with HindIII was included as a
standard. For Southern blotting, 10 μg DNA was digested overnight at 37°C with 20 units of restriction enzyme in 25 μl of the appropriate buffer provided by the supplier (Boehringer). The extent of digestion was determined by electrophoresis of a portion of the digested DNA (1/25th of the volume) on an agarose mini-gel, and the DNA was redigested overnight with an additional 5 units of enzyme where necessary. The digested DNA was then fractionated by electrophoresis on a 0.7% (wt/vol) agarose gel (13 × 18 cm) for 16 hours at 45 V. The fractionated DNA was denatured with alkali and transferred to a nylon membrane (Hybond-N, Amersham International) by capillary blotting in 20 x saline-sodium citrate buffer for a minimum of 18 hours.

DNA Probes

Two cDNA probes were prepared from plasmid pLDLR3, kindly provided by Dr. D. Russell, Dallas, Tex. The 1913 probe comprised a 1.9-kb BamHI fragment extending from the BamHI site in exon 10 to that in exon 18. The 1700 probe comprised a 1.7-kb HindIII/BglII fragment extending from the HindIII site in the polylinker of the vector to the BglII site in exon 12 (Figure 1).14 Exon-specific probes and a 720-bp fragment comprising exon 1 and the 5′ flanking sequences containing the promoter region for the gene were prepared by polymerase chain reaction (PCR)–dependent amplification as described below. All probes were purified by electrophoresis on low-melting-point agarose (Nusieve, FMC Biochemicals) and labeled with 32P-deoxycytidine triphosphate by random-primed synthesis.16

Gene Amplification

Fragments of the LDL receptor gene were amplified by PCR17 essentially as described previously8 with oligonucleotide probes located in the introns adjacent to the exon or pair of exons to be amplified.5 The 5′
**Figure 3.** Characterization of the \(\text{Bgl II}\) polymorphism in the low density lipoprotein receptor gene. Panel a: Southern blot of genomic DNA from members of the families of FH 120 and FH 118. Genomic DNA was digested with \(\text{Bgl II}\) or a combination of \(\text{Bgl II}\) and \(\text{EcoRV}\) as indicated and hybridized with the 720-bp probe to the promoter plus exon 1. Numbers below each lane refer to individuals identified in the pedigrees below. Panel b: Pedigrees of FH 120 and FH 118 showing inheritance of the \(\text{Bgl II}\) restriction fragment length polymorphism; "+" indicates the presence and "-" the absence of the cutting site. Those individuals whose DNA was available for study are identified by a number below the symbol. Shaded symbols represent individuals with a clinical diagnosis for heterozygous familial hypercholesterolemia (FH). Affected individuals in the family of FH 118 are heterozygous for a 3-bp deletion in exon 4; the mutation causing defective low density lipoprotein receptor function has not yet been identified in FH 120. ◊, Males; ○, females; ⊖, deceased.

**Results**

When genomic DNA from 200 apparently unrelated individuals with a clinical diagnosis of heterozygous (189) or homozygous (11) FH was analyzed by Southern blotting with cDNA probes specific for the LDL receptor gene, nine patients were found with an abnormal restriction fragment pattern characteristic of a major gene deletion or rearrangement (Figure 2). In three patients, digestion of genomic DNA with \(\text{Pvu II}\) followed by hybridization with a 1,913-bp cDNA probe encompassing exons 11–18 revealed an extra band (Figure 2a); also shown are the patterns obtained for three normolipemic individuals that demonstrate the common \(\text{Pvu II}\) polymorphism in intron 15 of the LDL receptor gene (Figure 2b). In one of these patients and in another seven patients, the restriction fragment pattern obtained after digestion of genomic DNA with \(\text{Bgl II}\) and hybridization with a 1,700-bp cDNA probe extending from exon 1 to exon 11 of the LDL receptor gene revealed extra bands (Figure 2c). The \(\text{Bgl II}\) blots were also hybridized with a 720-bp probe derived from exon 1 and the adjacent 5' flanking region (Figure 2d). As well as the expected band of 10 kb, a second band of approximately 13 kb was observed in approximately 13% of the individuals; two individuals with only the 13-kb band were identified. The expected 10-kb band was detected weakly when the blot was probed with the 1700 probe, but the new 13-kb band was obscured by the presence of the 13-kb band comprising exons 4–11. When DNA from individuals with the 13-kb band was digested with a combination of \(\text{EcoRV}\) and \(\text{Bgl II}\), in each case a single band of approximately 4.3 kb was observed (Figure 3a), suggesting that the \(\text{Bgl II}\) site at the 5' end of the promoter region was polymorphic (Figure 1).

Based on the observations that 112 individuals were homozygous for the common allele characterized by the 10-kb \(\text{Bgl II}\) fragment encompassing the promoter and exon 1, two were homozygous for the infrequent allele characterized by the 13-kb fragment, 17 individuals were heterozygous at this site, the calculated frequency of the rare allele in this group of FH patients was 0.08, and distribution of the alleles was in Hardy-Weinberg equilibrium. Mendelian inheritance of the infrequent allele was observed in two families, those of FH 118 and FH 120 (Figure 3b). In the family of FH 120 the infrequent allele cosegregated with hypercholesterolemia and a clinical diagnosis of heterozygous FH; in the
Table 1. Summary of Results of Southern Blotting to DefineExtent of Gene Deletions

<table>
<thead>
<tr>
<th>FH patient*</th>
<th>Enzyme digest†</th>
<th>Probe</th>
<th>Predicted bands§ (kb)</th>
<th>Extra bands$ (kb)</th>
<th>Conclusions and comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>FH 22/224/51</td>
<td>Bgl II</td>
<td>1700</td>
<td>9+13</td>
<td>12.0</td>
<td>Extra band not detected by probes to exons 3, 4, and 5+6; detected by probe to exon 7.</td>
</tr>
<tr>
<td>FH 22/224/51</td>
<td>Pvu II</td>
<td>1913</td>
<td>3.5+16.5/14</td>
<td>None</td>
<td>Bgl II site at intron 11/exon 12 intact.</td>
</tr>
<tr>
<td>FH 22/224</td>
<td>Kpn I/Xba I</td>
<td>1700</td>
<td>15 (Faint 9+6)</td>
<td>14</td>
<td>Cosegregation of FH 118 the infrequent allele with the occurrence of a 3-bp deletion in exon 4 of the LDL receptor gene that is known to cause defective LDL receptor function.</td>
</tr>
<tr>
<td>FH 51</td>
<td>Kpn I/Xba I</td>
<td>1700</td>
<td>15 (Faint 9+6)</td>
<td>19</td>
<td>Detection includes Xba I site in intron 1; probably &gt;10-kb deletion including promoter to intron 6.</td>
</tr>
<tr>
<td>FH 28/137</td>
<td>Bgl II</td>
<td>1700</td>
<td>9+13</td>
<td>12.5</td>
<td>Bgl II site at intron 11/exon 12 intact.</td>
</tr>
<tr>
<td>FH 28/137</td>
<td>Pvu II</td>
<td>1913</td>
<td>3.5+16.5/14</td>
<td>None</td>
<td>Detected by probes to exons 3 and 4 but not to exons 5+6 or 8; ~1-kb deletion including EcoRI site in exon 5. Confirmed by PCR across exons 4-6.</td>
</tr>
<tr>
<td>FH 28</td>
<td>EcoRI</td>
<td>Various</td>
<td>10</td>
<td>10.5</td>
<td>EcoRIV site in intron 14 intact.</td>
</tr>
<tr>
<td>FH 137</td>
<td>Bgl III</td>
<td>Exons 7 or 8</td>
<td>13</td>
<td>12.5</td>
<td>Extra band detected by probe to exon 8 but not by that to exon 7; ~1-kb deletion of exon 7.</td>
</tr>
<tr>
<td>FH 172</td>
<td>Pvu II</td>
<td>1913</td>
<td>3.5+14</td>
<td>9.5</td>
<td>Extra band not detected by probe to 5' end of exon 18, but band suggests Pvu II site in exon 18 probably intact.</td>
</tr>
<tr>
<td>FH 172</td>
<td>Bgl II</td>
<td>1913</td>
<td>21</td>
<td>10.5</td>
<td>~10.5-kb deletion between exons 12 and 18.</td>
</tr>
<tr>
<td>FH 172</td>
<td>EcoRV/Xba I</td>
<td>Exons 12-15</td>
<td>8.5</td>
<td>5.5</td>
<td>EcoRIV site in intron 14 intact.</td>
</tr>
<tr>
<td>FH 172</td>
<td>Kpn I</td>
<td>Exons 12-15</td>
<td>9+11</td>
<td>None</td>
<td>Kpn I site in intron 14 intact, but intron 15 missing. Deletion extends from mid intron 14 to mid exon 18.</td>
</tr>
<tr>
<td>FH 218</td>
<td>Bgl III</td>
<td>1700</td>
<td>9.5+13</td>
<td>~23</td>
<td>Detected by 1913, exons 4 and 5+6 but not exon 3. Deletion of ~11-kb including Bgl II site at intron 11/exon 12.</td>
</tr>
<tr>
<td>FH 218</td>
<td>Kpn I</td>
<td>Exons 12-15</td>
<td>19+9</td>
<td>17</td>
<td>Exon 14 missing, but exon 15 partially present. Deletion extends from intron 6 to intron 14.</td>
</tr>
<tr>
<td>FH 200</td>
<td>Bgl III</td>
<td>1700</td>
<td>9.5+13</td>
<td>&gt;23</td>
<td>Detected by 1913, exons 4 and 5+6 but not exon 3. Deletion of ~10-kb including Bgl II site at intron 11/exon 12.</td>
</tr>
<tr>
<td>FH 200</td>
<td>Kpn I</td>
<td>Exons 12-15</td>
<td>19+9</td>
<td>~18</td>
<td>Similar to FH 218, but deletion slightly smaller.</td>
</tr>
<tr>
<td>FH 185</td>
<td>Bgl II</td>
<td>1700</td>
<td>9.5+13</td>
<td>~17</td>
<td>Bgl II site at intron 11/exon 12 intact.</td>
</tr>
<tr>
<td>FH 185</td>
<td>Pvu II</td>
<td>1913</td>
<td>3.5+14+16.5</td>
<td>None</td>
<td>Deletion extends from intron 11/exon 12 intact.</td>
</tr>
<tr>
<td>FH 185</td>
<td>EcoRI</td>
<td>1700</td>
<td>10+1.7+8</td>
<td>4+(12)</td>
<td>12 kb detected by probes to exons 3, 4, and 5+6 but not exon 8 (probably from partial cleavage of EcoRI site in exon 5); 4 kb detected by probe to exon 8, but not exons 3-6.</td>
</tr>
<tr>
<td>FH 185</td>
<td>Sac I/EcoRV</td>
<td>1700</td>
<td>15</td>
<td>None</td>
<td>Gene normal between exons 2 and 6.</td>
</tr>
<tr>
<td>FH 185</td>
<td>Kpn I/Xba I</td>
<td>1700</td>
<td>15 (Faint 9+6)</td>
<td>19</td>
<td>Duplication (4 kb) of exons 7+8 in intron 6. Extra 4-kb band on EcoRIV blot from duplication of site in intron 6.</td>
</tr>
</tbody>
</table>

*The number is an arbitrary identification assigned to the patient for this study.
†Genomic DNA from each individual was digested with the restriction enzyme indicated and hybridized with 32P-labeled probe as described in "Methods."
‡Bands predicted from the restriction enzyme map of the low density lipoprotein receptor gene (see Figure 1).
§Extra bands that were not detected on blots of DNA from unaffected individuals.
||Data shown in Figure 2 or 4.
FH, familial hypercholesterolemia; PCR, polymerase chain reaction.

The family of FH 118 the infrequent allele cosegregated with the occurrence of a 3-bp deletion in exon 4 of the LDL receptor gene that is known to cause defective LDL receptor function. Apart from this polymorphism, no unexpected bands were observed for any individual on blots of Bgl II–digested DNA hybridized with the promoter probe.

To analyze the nature of the gene defect in each case, further enzyme digestions of genomic DNA were performed and the blots hybridized with exon-specific probes as described in Table 1. Southern blots showing a characteristic pattern of bands for each deletion are shown in Figure 4.

Discussion

Of the 200 FH patients in this sample from lipid clinics in the London area, nine have been found to be heterozygous for a defective LDL receptor gene in which a major deletion or gene rearrangement was detectable by Southern blotting. The data are summarized in Table 2. Because 11 of the 200 patients are clinically homozygous and at least seven of these are
compound heterozygotes with two different defective alleles, the nine mutant alleles represent approximately 4.5% of the total. This value is similar to that found when other groups of FH patients in the United Kingdom21 or Canada19 have been screened for major gene deletions or rearrangements in this way. However, in a Dutch population, 17.5% of 53 FH patients have recently been found to have a mutant allele that could be detected by Southern blotting, but this rather high frequency resulted from the presence of one common deletion in more than half of these individuals.22

Only one of the deletions identified in our study was present in more than one of the 200 unrelated individuals, but a number of them have been observed previously in FH patients of British or European ancestry. For example, the gene defect identified in two patients in this study (FH 22 and 224), one of whom is a compound heterozygote, was a 10-kb deletion that appears to be identical to that found in a Canadian patient (identified as FH 49) characterized by Langlois and coworkers.19 Of the six patients with deletions in the LDL receptor gene identified by these authors, five were apparently of English or Irish origin, and thus, it is likely that all of the individuals with this deletion have inherited the same mutant allele from a common ancestor. The phenotype of cells expressing this mutant allele has not been determined, but even if a receptor protein with such a large deleted fragment was synthesized and processed, then it would at best be markedly defective in its ability to bind LDL.

The deletion of 1 kb encompassing exon 5 that we have observed in a single individual (FH 28) has also been described previously in two patients of European origin, one English21 and the other French.20 The phenotype of the LDL receptor protein in cells expressing this mutant allele has been shown to be binding defective, with LDL binding being more severely affected than binding of lipoproteins containing apolipoprotein E.20 It is also probable that the 1-kb deletion of exon 7 that we have observed in a single patient (FH 137) is the same as that described in a patient of English origin by Horsthemke et al.21 Cultured cells are not available from FH 137, but because the donor splice sites for exons 6 and 7 are in frame,14 the resultant abnormal mRNA should code for a receptor protein that has the first growth factor-like repeat A in domain 2 missing but is otherwise normal. By analogy with a similar mutation introduced by site-directed mutagenesis and expressed in heterologous cells, the phenotype of such a receptor would be mildly binding defective.25

The other five defective LDL receptor genes identified by Southern blotting in this sample of FH patients in the United Kingdom do not appear to have been described previously, although there are similarities
### Table 2. Summary of Deletions and Rearrangements

<table>
<thead>
<tr>
<th>FH patient</th>
<th>Plasma cholesterol (mmol/L)</th>
<th>Diagnosis</th>
<th>Ethnic origin</th>
<th>Mutation</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>FH 22</td>
<td>10.0</td>
<td>Htz</td>
<td>UK</td>
<td>Approximately 10-kb deletion of exons 2–6</td>
<td>Same as FH 49 described by Langlois et al(^{19})</td>
</tr>
<tr>
<td>FH 224</td>
<td>26.0</td>
<td>Compound Htz*</td>
<td>UK</td>
<td>&gt;10-kb deletion of promoter to exon 6</td>
<td>Not previously described</td>
</tr>
<tr>
<td>FH 51(\dagger)</td>
<td>16.3</td>
<td>Compound Htz(\ddagger)</td>
<td>UK</td>
<td>Approximately 1-kb deletion of exon 7</td>
<td>Same as FH Paris(^{20}) and FH London (^{21})</td>
</tr>
<tr>
<td>FH 28(\dagger)</td>
<td>8.9</td>
<td>Htz</td>
<td>UK</td>
<td>Approximately 1-kb deletion of exon 7</td>
<td>? Same as KL(^{21})</td>
</tr>
<tr>
<td>FH 137(\dagger)</td>
<td>8.7</td>
<td>Htz</td>
<td>UK</td>
<td>Approximately 1-kb deletion of exon 7</td>
<td>Not previously described, but common deletion of exons 7 and 8 in Dutch FH patients(^{22})</td>
</tr>
<tr>
<td>FH 185</td>
<td>8.3</td>
<td>Htz</td>
<td>Polish</td>
<td>Duplication of exons 7 and 8 in intron 6</td>
<td>Not previously described</td>
</tr>
<tr>
<td>FH 200</td>
<td>8.3</td>
<td>Htz</td>
<td>Asian Indian</td>
<td>Approximately 11-kb deletion of exons 7–14</td>
<td>Not previously described</td>
</tr>
<tr>
<td>FH 218</td>
<td>12.0</td>
<td>Htz</td>
<td>UK</td>
<td>Approximately 10-kb deletion of exons 7–14</td>
<td>? Similar to FH Osaka (^{23})</td>
</tr>
<tr>
<td>FH 172(\dagger)</td>
<td>15.9</td>
<td>Compound Htz</td>
<td>UK</td>
<td>Approximately 10.5-kb deletion of exons 15–18</td>
<td>Not previously described</td>
</tr>
</tbody>
</table>

*Htz, heterozygote; compound Htz, clinical diagnosis of homozygous familial hypercholesterolemia (FH) but two different mutant alleles; PCR, polymerase chain reaction.*

\(\dagger\)The other mutant allele has not been characterized.

\(\ddagger\)The other mutant allele in this individual has a point mutation (Glu\(\rightarrow\)Lys).\(^{24}\)

with deletions found in FH patients elsewhere. For example, the deletions in FH 200 and FH 218 in our study that both encompass exons 7–14 are similar to a deletion in a Japanese patient described by Miyake et al.\(^{23}\) The deletion in this patient was believed to be caused by misalignment of Alu sequences in intron 6 and intron 14, and although we have not cloned the deletion joint in our patients, it is likely that the deletion in FH 200 is identical to that in the Japanese patient, while that in FH 218 involves different Alu repeats and results in a slightly larger deletion. If the primary transcripts of RNA are spliced to produce a receptor protein that lacks domain 2 but is otherwise normal, as has been observed in cells from the Japanese patient,\(^{23}\) then the mutant alleles in FH 200 and FH 218 will result in the same recycling-impaired phenotype.

The large deletion at the 5' end of the gene that we have observed in FH 51 is superficially similar to that described recently in an Italian patient by Lelli et al\(^{26}\) (identified as FH 44 or FH Bologna). However, the 5'...

### Table 3. Summary of Mutations in the LDL Receptor Gene in Familial Hypercholesterolemia in the United Kingdom

<table>
<thead>
<tr>
<th>Type of mutation</th>
<th>No. of unrelated patients</th>
<th>Comments</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deletions/rearrangements detected by Southern blotting</td>
<td>9*</td>
<td>4/9 Same as and 3/9 similar to previously detected mutations</td>
<td>This article</td>
</tr>
<tr>
<td>Pro(\rightarrow)Leu</td>
<td>5(\dagger)</td>
<td>Recurrent mutation</td>
<td>11</td>
</tr>
<tr>
<td>Glu(\rightarrow)Lys</td>
<td>5*</td>
<td>Probably same allele</td>
<td>24</td>
</tr>
<tr>
<td>Exon 4 mutations: deletion of Gly(\rightarrow)Arg</td>
<td>6(\ddagger)</td>
<td>Same allele as FH Piscataway</td>
<td>3</td>
</tr>
<tr>
<td>New deletion of 2 bp in codons 206/207</td>
<td>5</td>
<td>Same allele</td>
<td>Unpubl obs</td>
</tr>
<tr>
<td>New Cys(\rightarrow)Stop</td>
<td>1$</td>
<td></td>
<td>Unpubl obs</td>
</tr>
<tr>
<td>Asp(\rightarrow)Glu</td>
<td>3</td>
<td>Same as FH Maine/Afrikaner 1</td>
<td>6</td>
</tr>
<tr>
<td>Ser(\rightarrow)Leu</td>
<td>1$</td>
<td>Same as FH Puerto Rico, but recurrent mutation?</td>
<td>31</td>
</tr>
<tr>
<td>Total</td>
<td>35 (17% of mutant alleles)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*One clinically homozygous patient is a compound heterozygote in whom one allele has a deletion and the other a point mutation (Glu\(\rightarrow\)Lys).*

\(\dagger\)One of the patients is a compound heterozygote in whom the other mutant allele has not yet been characterized.

\(\ddagger\)Patients (4, three of six) also heterozygous for Bgl II restriction fragment length polymorphism in 5' flanking region.

LDL, low density lipoprotein; FH, familial hypercholesterolemia; unpubl obs, unpublished observations.
limit of the deletion must be different in our patient, as we did not observe the additional band of 7.5 kb on the EcoRI blot, and the abnormal band on the Bgl II blot was smaller than that observed with FH 51; the deletion in FH 51 may extend farther at the 5' end of the gene and would undoubtedly result in a null phenotype.

The remaining deletion observed in this sample of 200 patients, that of 10.5 kb encompassing exons 14–18 in FH 172, has apparently not been observed previously. Several mutant LDL receptor genes have been described in which exons 16–18 are deleted, namely the Finnish mutation,37 FH Osaka-2 (FH 781 in Lehrman et al28), and FH Rochester (FH 274 in Lehrman et al29), but the restriction fragment patterns obtained with FH 172 differ, and blotting with exon-specific probes confirmed that exon 15 is also deleted in this patient. Although we have not cloned the deletion joint, it is likely that misalignment of Alu sequences in intron 14 and exon 18 is responsible for this deletion. Preliminary studies (V. Gudnason et al, unpublished observations) have shown that the mutant allele with this deletion in cultured cells from FH 172 produces a truncated receptor protein that is not processed to a mature form and resembles the abnormal protein detected in cells with the Lebanese mutation, where a stop codon has been introduced into exon 14 as the result of a point mutation in codon 660.4

The gene defect in the final patient in this study (FH 185) was a duplication of exons 7 and 8 inserted in intron 6, a defect that has not been described previously. However, it is of interest that a 4-kb deletion of exons 7 and 8 has been observed as a frequent cause of the disease in Dutch FH patients,22 and it is tempting to speculate that this allele in FH 185 represents the complementary chromosome that could be formed when such a deletion occurs as a result of unequal crossing over. The phenotype has not yet been determined for this mutation.

In addition to the mutations described above, a new restriction fragment length polymorphism for Bgl II in the 5' flanking region of the gene was detected as a heterozygous trait in 13% of the patients. The frequency of the rare allele, characterized by a 13-kb Bgl II fragment compared with 10 kb for the common allele, was 0.08 in this group of FH patients. Although we have not attempted to screen normal individuals for this restriction fragment length polymorphism, it is unlikely that it represents a deleterious mutation responsible for FH in these patients, as it was found in some heterozygous FH individuals in whom a point mutation or a small deletion in the gene that is known to cause defective LDL receptor function had also been identified (Table 3).

In summary, of the six deletions found in patients of apparently British ancestry in our sample of 200 patients, three have been identified previously in FH patients of UK origin. The remaining three mutations have not been described but are deletions in regions that seem to be susceptible due to the presence of numerous Alu sequences.3 The low frequency of each individual deletion in the LDL receptor gene as a cause of FH makes it difficult to predict whether the deletions in this population of 200 FH patients are representative of those that will be found in other FH patients in the United Kingdom. This low frequency of each individual deletion is a little surprising, as this same group of 200 patients has now been screened for a number of known mutations in the LDL receptor gene (V. Gudnason et al, unpublished observations, and References 24 and 30), and as summarized in Table 2, the majority of these have been observed in more than one unrelated patient. Indeed, several mutations, including Pro61—>Leu,30 Glu95—>Lys,31 and the deletions of Gly197 and of 2 bp at the 3' end of exon 4 (V. Gudnason et al, unpublished observations), are present in 2–3% of the patients. The available data suggest that at least some of these more frequently occurring mutant alleles have all been inherited from a single ancestor of English origin and that this value of 2–3% could represent the highest frequency that can be expected for any mutation causing FH in the United Kingdom. However, the 200 patients in this study have been drawn from the population around London and the southeast of England and may comprise a more heterogeneous group than would be encountered elsewhere in the United Kingdom. Indeed, one of the point mutations (Glu95—>Lys) has been found at a much higher frequency in a sample of FH patients attending a lipid clinic in Manchester, where the phenotype may have been more stable over the last century.24

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