Identification of deletions in the human low density lipoprotein receptor gene

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SUMMARY DNA samples from 70 unrelated UK patients with heterozygous familial hypercholesterolaemia were screened by Southern blot hybridisation with a 5' fragment of the human low density lipoprotein (LDL) receptor cDNA. In the majority of cases, the restriction fragment pattern of the LDL receptor gene was indistinguishable from that observed in normal subjects. However, three patients were found to have a deletion of approximately 1 kb in the central portion of the gene. Mapping experiments indicated that in two patients a similar deletion had occurred that includes all or part of exon 5, and in the third patient a deletion has occurred that includes exon 7. Taking into account our previously described patient with a deletion in the 3' part of the gene, this means that in four out of 70 UK patients with familial hypercholesterolaemia (6%), the defect is caused by a detectable deletion of part of the coding portion of the low density lipoprotein receptor gene.

Familial hypercholesterolaemia (FH) is caused by a reduction in cellular low density lipoprotein (LDL) uptake due to defects in the LDL receptor.^{1,2} The disease is clinically characterised by raised serum and LDL cholesterol levels, tendon xanthomata in some cases, and increased risk of myocardial infarction after the age of 35 years.³⁻⁵ With one person in 500 carrying a mutant LDL receptor gene, FH contributes significantly to the incidence of coronary artery disease. Recently, the full length cDNA and the entire LDL receptor gene have been cloned.^{6,7} So far three deletions,⁸⁻¹⁰ one small insertion, and one point mutation¹¹ have been reported. All these mutations are located in the 3' third of the 45 kb gene. Here we have used a 5' cDNA fragment to search for gene deletions or rearrangements in the 5' part of the gene.

Materials and methods

Blood samples were collected from heterozygous FH patients attending lipid clinics at the Metabolic Unit at St Mary's, St George's, St Thomas's, and

Hammersmith Hospitals. Lipoproteins were analysed using standard techniques and patient criteria were those previously described.¹² Briefly, patients have plasma LDL cholesterol levels above 4 mmol/l, tendon xanthomata in some cases, and family history of premature myocardial infarction of type IIa hyperlipidaemia or both.

GENE PROBES FOR THE LDL RECEPTOR GENE

The full length cDNA clone pLDLR-3 was a kind gift from Dr D W Russell, Dallas, USA. Plasmid DNA was prepared by the alkaline lysis method and was then digested with *Hind*III and *Bgl*II (Anglian Biotechnology). The resulting 1.7 kb cDNA fragment (nucleotides 1 to 1706) was isolated on a 1% low gelling temperature agarose gel (BioL) and purified as described.¹⁴ This probe was then labelled to a specific radioactivity of greater than 10⁶ cpm/μg by random oligonucleotide priming (Pharmacia Biochemicals) using [³²P] dCTP at 800 Ci/mmole (Amersham).¹⁵

DNA ANALYSIS

Blood samples were collected in EDTA tubes and frozen at -20°C. Total genomic DNA was prepared from leucocytes by a Triton X 100 lysis method. DNA (5 μg) was digested with restriction enzymes and separated on a 0.7% agarose gel, and transferred to

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Zetabind filters (AMF Cuno). Hybridisation and autoradiography were as described previously.⁹

Results

DNA samples from 70 unrelated FH patients were digested with *Bgl*II and analysed by Southern blot hybridisation with the 5' cDNA fragment. This probe hybridises to exons 1 to 11, which are contained in *Bgl*II fragments of 10 kb, 13 kb, and 9.5 kb. An example of six digests is shown in fig 1. Since the most 5' 10 kb fragment contains only one exon, the hybridisation signal is much fainter than with the other bands and can be seen only after very long exposures (not shown). Three of the patients (PO, JA, and KL), who are heterozygous for FH, were found to have an additional fragment of approximately 12 kb.

To determine whether this pattern was the product of a rare *Bgl*II polymorphism or of deletions in the gene, we performed *Eco*RI and *Eco*RI/*Bam*HI single and double digests. Southern blot filters were hybridised with the 1.7 kb 5' cDNA probe (fig 2) and the pattern of fragments observed was compared with the expected fragments predicted from the restriction map of the LDL receptor gene⁷ (fig 3). Samples from the three patients also contain additional fragments with these enzymes, indicating that the patterns seen are due to gene deletions rather than to a *Bgl*II polymorphism. Patients PO and JA show the same pattern: the probe detects a larger 11 kb *Eco*RI fragment, while the 10 kb and 1.7 kb fragments are present at only half the normal intensity. This indicates that the deletion in one allele of both these patients includes the *Eco*RI site in exon 5 and suggests that all or part of exon 5 has been deleted. Thus, the 11 kb fragment is a product

FIG 2 Southern blot hybridisation of (A) *Eco*RI and (B) *Eco*RI/*Bam*HI cleaved genomic DNA from patients PO, JA, and KL and one normal subject N.

of the normal 10 kb plus the 1.7 kb fragments minus the deletion.

The pattern of hybridising fragments in DNA from patient KL indicates that the deletion is in a different region of the gene from patients PO and JA. In patient KL, the probe detects an additional 8 kb *Eco*RI fragment, which could arise from a deletion of roughly 1 kb from the normal 9 kb fragment. In the *Eco*RI/*Bam*HI double digests, the hybridisation intensity of the 3.2 kb band is reduced, while the intensity of the 2.2 kb band is increased. This indicates that there has been a deletion of roughly 1 kb from the 3.2 kb fragment containing exon 7, resulting in the detection of a fragment of 2.2 kb.

Discussion

Of the 70 patients with FH that we have examined, three have a detectable deletion in the central part of the gene. From our preliminary mapping experiments it is possible to estimate the extent of these deletions. For patients PO and JA, the deletion has removed all or part of exon 5 which codes for the final part of the LDL receptor ligand binding domain, and for patient KL, the deletion affects exon 7, which codes for the first part of the 'stem' region of the receptor.^{6,7} These deletions clearly interrupt normal LDL receptor function and cause FH. If, however, a deletion has excised an entire

FIG 1 Southern blot hybridisation of *Bgl*II cleaved genomic DNA from patients PO, JA, and KL and normal subjects 1, 2, and 3. Fragment sizes were calculated from molecular size standards of bacteriophage λ DNA cleaved with *Hind*III.

FIG 3 Map of LDL receptor gene showing the positions of the deletions relative to the normal gene. The restriction enzyme sites shown are BglII (G), BamHI (B), and EcoRI (E). Patients PO and JA lack the EcoRI site normally present in exon 5 (shown as X). The relative positions of exons 2 to 12 are shown together with the sizes in kb of the normal BglII and EcoRI fragments.

exon rather than just a portion of one, then it is possible that the reading frame of the mRNA is not altered when the introns are spliced out. In the LDL receptor gene, exon 4 ends with the first base of a codon triplet, while both exon 5 and exon 6 start with two bases to complete a codon triplet.⁶ It is thus possible that in patients PO and JA, with a deletion of exon 5, exon 4 may be spliced onto exon 6 during processing of the RNA transcript. Translation of this mRNA should result in the production of a receptor protein lacking amino acids 211 to 292.

Similarly, in patient KL, where exon 7 is affected, exons 6 and 8 could be spliced together to form an mRNA that would be translated to give a protein lacking amino acids 293 to 374. It will be possible to test these hypotheses by culturing fibroblasts from the patients and examining the processing and function of the receptor proteins using Western blotting techniques.

At the resolution of the mapping experiments we have carried out, the deletion in patients PO and JA appears to be identical. A more detailed comparison will require the isolation and the sequencing of this allele of the genes from the two patients. It is possible that the deletion may have occurred independently on two occasions, or more likely that the patients have both inherited the defective gene from a common ancestor. To date we have not been able to determine a shared family history for these patients.

We have now detected four deletions of the LDL receptor gene among the 70 FH patients we have screened, the three reported here together with the previously reported deletion in the 3' region of the gene.⁹⁻¹⁷ Such deletions thus appear to be a significant cause of FH (~6%) in the UK population. This high frequency of LDL receptor gene deletions can perhaps be explained by the frequent occurrence of highly repetitive Alu repeat sequence present in the intervening sequences and 3' untranslated region of this gene.^{8-10,17} Mechanisms of recombination due to either homologous or non-homologous pairing of Alu repeat sequence, followed by crossing over during meiosis, have been invoked to explain the other deletions in the LDL receptor. Sequencing experiments would reveal whether these three deletions in the central part of the gene have arisen in a similar manner.

For the 6% of patients with a detectable deletion of the LDL receptor gene, it will be possible to carry out direct presymptomatic diagnosis for familial hypercholesterolaemia.

At present, until we know more about the nature of the defects at the DNA level which cause FH, presymptomatic DNA diagnosis for the remainder of the patients will require the use of restriction fragment length polymorphisms in family studies.

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