# Characterization of mutations in the low density lipoprotein (LDL)-receptor gene in patients with homozygous familial hypercholesterolemia, and frequency of these mutations in FH patients in the United Kingdom

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**Abstract** Mutations in the gene for the low density lipoprotein (LDL) receptor have been identified in 15 patients with homozygous familial hypercholesterolemia (FH). Five patients are homozygous at the LDL-receptor locus; their mutant alleles include Glu387Lys and Pro664Leu in patients of Asian-Indian descent, Cys292Stop in a Greek Cypriot, Cys281Trp in a Turkish patient, and Gln540Stop in a West Indian. The other 10 patients (9 of apparently British ancestry) are compound heterozygotes. Mutations have been identified in 18 of 20 possible alleles, including Glu80Lys (2 patients), Pro664Leu (3 patients), Asp69Gly, Cys176Arg, Cys227Tyr, Ser265Arg, Asp280Ala, Asp283Glu, Arg329Pro, Asp461Asn, Leu578Ser, a single bp deletion in exon 15, a 21 bp duplication of codons 200–206 and two large deletions. The seven mutations underlined above have not been described previously. The two uncharacterized mutant alleles fail to produce detectable amounts of mRNA. LDL-receptor activity in cultured cells from 13 of the 15 homozygous patients varied from undetectable to about 30% of normal, but there was no correlation between LDL-receptor activity and the untreated plasma cholesterol concentration in these patients. When genomic DNA from 295 patients with a clinical diagnosis of FH was screened for 29 mutations found in these and other FH patients of British ancestry, most were identified in only one or a few individuals. Four patients heterozygous for the Asp461Asn allele showed a wide range of clinical manifestations. These observations confirm the striking heterogeneity underlying FH in most populations and demonstrate the variability in phenotype between patients with the same mutation.—**Webb, J. C., X-M. Sun, S. N. McCarthy, C. Neuwirth, G. R. Thompson, B. L. Knight, and A. K. Soutar.** Characterization of mutations in the low density lipoprotein (LDL)-receptor gene in patients with homozygous familial hypercholesterolemia, and frequency of these mutations in FH patients in the United Kingdom. *J. Lipid Res.* 1996. **37:** 368–381.

**Supplementary key words** genotype • phenotype • automated DNA sequencing • genetic screening • heterozygous familial hypercholesterolemia

Familial hypercholesterolemia (FH) is a relatively common co-dominantly inherited disorder of plasma lipoprotein metabolism that is caused by defects in the gene for the low density lipoprotein (LDL) receptor (1). The LDL receptor plays a central role in the pathways involved in cholesterol transport in plasma and defects in its function affect not only the specific uptake and catabolism of LDL, the major carrier of cholesterol in the circulation, but also its rate of production in plasma. The raised concentration in plasma of LDL-cholesterol that invariably accompanies defective LDL-receptor function, at least in western societies, causes accumulation of cholesterol in the peripheral tissues in the form of xanthoma and leads to accelerated atherosclerosis. Patients with heterozygous FH are at increased risk for premature coronary heart disease (CHD) in early middle age, while homozygous FH individuals frequently suffer fatal CHD by their third decade (1).

Considerable variation occurs in the clinical expression of FH that has been suggested to be the result of genetic heterogeneity in the LDL-receptor defect, especially in homozygous FH patients (2). The LDL receptor is a multifunctional protein whose structure comprises

Abbreviations: FH, familial hypercholesterolemia; LDL, low density lipoprotein; CHD, coronary heart disease; RT-PCR, reverse transcription of RNA and amplification of the cDNA by the polymerase chain reaction.

[1]This paper is dedicated to the memory of Sue McCarthy, who died suddenly on September 29, 1995.

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several well-defined domains that appear to fulfil separate roles in the pathway of receptor-mediated uptake and catabolism of lipoproteins, and there is already clear evidence that mutations in parts of the gene coding for different domains can affect different aspects of receptor function to different extents. For example, movement of newly synthesized receptors to the cell surface, ligand binding, internalization of receptor-ligand complexes or recycling of receptor to the cell surface can each be affected by amino acid substitutions in different domains (3). However, a relationship between the nature of the mutation and the severity of the disorder has not been clearly established for heterozygous individuals and our observation that the obligate heterozygous parents of Chinese homozygous FH patients have only mild hypercholesterolemia and none of the clinical signs usually associated with heterozygous FH suggests that other genetic or environmental factors may also be important (4).

Many different mutations in the gene can cause FH (5), but with the exception of those genetically isolated populations in which a founder gene effect is apparent, until recently there has been no systematic analysis of the mutations underlying FH in more typical, heterogeneous populations such as that in the UK. In collaboration with Humphries and coworkers, we have assembled DNA samples from a group of 200 patients with a clinical diagnosis of FH who have attended lipid clinics in London; the results of screening this group of patients for a small number of mutations in the LDL-receptor gene have been described previously (6–10). We have also had the opportunity to study a number of homozygous FH patients who have been referred during the past 25 years to the Hammersmith Hospital Lipid Clinic for treatment or counselling, nine of whom are of apparently British ancestry with no apparent consanguinity in their pedigree. It seemed likely that the mutations responsible for FH in these individuals would be those that are most common in the UK population. In this study, therefore, we have characterized the mutant LDL-receptor alleles in these FH homozygotes and have then determined their frequency in the group of 200 FH patients and in an additional group of 95 well-characterized heterozygous FH patients attending the Hammersmith Hospital Lipid Clinic. Knowledge of the mutations underlying the defect in FH is an aid for unambiguous diagnosis within affected families and enables advice to be given at an early age. When sufficient patients with the same mutation have been identified, it will also make it possible to compare the clinical phenotype of groups of patients with different mutations so as to examine factors that influence the severity of the disorder.

## METHODS

### FH patients

Clinical details of the 15 homozygous FH patients, 12 of whom have been described elsewhere (8, 11–16), are shown in **Table 1.** In all cases, the diagnosis of homozygous FH was based on a grossly increased plasma cholesterol concentration and the presence of xanthomata in childhood and cardiovascular involvement by puberty

TABLE 1. Clinical details of patients with homozygous familial hypercholesterolemia

| Patient | Sex | Current Age or at Death[a] | Serum Cholesterol[b] | Cardiovascular Involvement | Non-pharmacological Treatment | Reference |
|---|---|---|---|---|---|---|
| | | *yr* | *mmol/l* | | | |
| 1 | F | 38 | 27 | Ao. stenosis, 3V CAD | Heart/liver transplant | 11 |
| 2 | M | 30[a] | 20.1 | Ao. stenosis, 3V CAD | Apheresis, CABG | 12 |
| 3 | M | 31[a] | 20.7 | Ao. stenoisis, 3V CAD | Apheresis, AVR, CABG | 12 |
| 4 | M | 7 | 26.8 | Normal angiogram | Apheresis | 8 |
| 5 | F | 45 | 15.6 | Ao. stenosis, 3V CAD | AVR, CABG, portocaval shunt | 12 |
| 6 | F | 28 | 17.1 | Ao. stenosis, carotid disease | None | 13 |
| 7 | F | 17 | 18.7 | Ao. stenosis | Apheresis | – |
| 8 | M | 19[a] | 25.2 | Ao. stenosis, 3V CAD | AVR, CABG | 12 |
| 9 | F | 14 | 22.0 | Normal angiogram, LVH on ECG | None | – |
| 10 | F | 12 | 28.7 | Aortic & carotid atherosclerosis | Apheresis | 14 |
| 11 | F | 23[a] | 26 | Ao. stenosis, 3V CAD | Apheresis, CABG | 12 |
| 12 | M | 31 | 22.3 | Ao. stenosis, 3V CAD | Apheresis, CABG | 12 |
| 13 | M | 26 | 24.4 | Ao. stenosis, carotid disease | Apheresis | 15 |
| 14 | M | 35 | 20.6 | Ao. stenosis, 3V CAD | None | 16 |
| 15 | F | 10[c] | 30.6 | Ischemic ECG | Unknown | – |

Abbreviations: Ao., aortic; AVR, aortic valve replacement; CABG, coronary artery bypass graft; LVH, left ventricle hypertrophy; 3V CAD, three vessel coronary artery disease.

[a]Age at death.

[b]Highest recorded value.

[c]Status unknown.



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in the proband, together with hypercholesterolemia in both parents. The group of 200 FH patients attending Lipid Clinics in the London area have been described previously (7); the additional 95 patients attended the Lipid Clinic at the Hammersmith Hospital during the past 2 years and have been given the diagnosis of definite or possible heterozygous FH according to the criteria laid down by the Simon Broome Register Group (17). None of the homozygous FH patients were carriers of the apoB-3500 mutation (18), and carriers of this allele were excluded from the two large patient groups.

### Identification of mutations in the LDL-receptor gene

DNA was isolated from whole frozen blood or from cultured skin fibroblasts or transformed lymphoblasts from the homozygous FH patients and stored frozen at -20°C. Amplification of each exon of the LDL-receptor gene and direct nucleotide sequencing of the PCR products was carried out as described previously (8). Single base changes were confirmed, where possible, by digestion of the amplified DNA product with restriction enzymes and by analyzing the DNA from affected and unaffected relatives of the proband. In some cases, PCR products were subcloned into plasmid pGEM-T (Promega Ltd.) and the nucleotide sequence of the insert was determined by automated cycle sequencing of miniprep DNA (PRISM™ Ready Reaction DyeDeoxy™ Terminator Cycle Sequencing Kit; Applied Biosystems) with primers complementary to sequences in the plasmid (either T7 or SP6 promoter) and following the supplier's protocol.

### Screening for mutations

DNA from each member of the two large groups of

TABLE 2. Mutations in the LDL-receptor gene in patients with homozygous FH in the UK

| Patient | Ethnic Origin | Genotype | Mutation | LDL-R activity[d] |
|---|---|---|---|---|
| | | | | *% of normal* |
| 1 | English | ab | a Δ exons 2–6 | (5–15)[g] |
| | | | b Leu578Ser | |
| 2 | English | ab | a Glu80Lys | 10–15[e] |
| | | | b Δ 5′-exon 6[a] | |
| 3 | English | ab | a Asp69Gly | 25–30[e] |
| | | | b Asp283Glu | |
| 4 | English/Irish | ab | a Glu80 Lys[a] | 25–30[e] |
| | | | b 1bp Δ in exon[a] | |
| 5 | English/Greek | ab | a Asp280Ala[b] | (5–15)[g] |
| | | | b Ser265Arg | |
| 6 | English | ab | a Cys227Tyr[b] | 10–15[f] |
| | | | b Arg 329Pro[b] | |
| 7 | English | ab | a 21pb Δ exon 4 | <2[f] |
| | | | b Asp461Asn[b] | |
| 8 | English | ab | a Pro664Leu | 15[f] |
| | | | b null, no mRNA | |
| 9 | English | ab | a Pro664Leu | 20[f] |
| | | | b Cys176Arg[b] | |
| 10 | Indian Asian | ab | a Pro664Leu | no cells |
| | | | b null, no mRNA | |
| 11 | Greek Cypriot | aa | a Cys292Stop | <1[e] |
| 12 | Indian Asian | aa | a Pro664Leu[a] | 20–25[e] |
| 13 | Indian Asian | aa | a Glu387Lys[b,c] | 5–10[f] |
| 14 | West Indian | aa | a Gln540Stop[a] | <1[e] |
| 15 | Iraqi | aa | a Cys281Trp[b] | no cells |

[a]Mutations described previously in these patients (7, 8, 16, 20).
[b]Previously undescribed mutations.
[c]Recurrent mutation (also observed in heterozygous patient of UK origin, Sun et al., unpublished).
[d]Saturable maximum degradation of $^{125}$I-labeled LDL by cultured skin fibroblasts[e] or Epstein-Barr-Virus-transformed lymphoblasts[f]
[g]Values from Hobbs et al. [5].



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patients was diluted to give approximately 50 μg/ml in sterile water and plated out in 96-well microtitre plates. Amplification of appropriate exons was also carried out in Hybaid microtitre plates in a final volume of 25 μl in a Hybaid Omnigene programmable thermal block; wells containing no DNA were always included as a control for monitoring contamination of the PCR reaction. PCR products were analyzed by electrophoresis on 1.0–1.5% (w/v) agarose gels and visualized with ethidium bromide.

Restriction enzyme digestion of PCR products was carried out in microtitre plates with 8 μl of the PCR reaction mix in a final volume of 15 μl of buffer recommended by the manufacturer for the particular enzyme used. Restriction enzyme digestion products were analyzed by electrophoresis, either on 1.5–2.0% agarose gels stained with ethidium bromide (for fragments > 100 bp) or on 8% non-denaturing polyacrylamide gels stained with either ethidium bromide or with silver (for fragments < 100 bp).

Detection of heteroduplexes or minor deletions in PCR products was carried out by electrophoresis on non-denaturing polyacrylamide gels (4–8%, depending on fragment size) stained with ethidium bromide.

Hybridization with allele specific oligonucleotides to detect single base changes was carried out essentially as described previously (8); briefly, 5 μl of PCR product was diluted with 200 μl with 15 × SSC, and 100 μl of this was loaded in duplicate onto nylon membranes by means of a vacuum slot-blotting apparatus. DNA was fixed to the blots by UV irradiation and the membranes were prehybridized in hybridization buffer (5 × SSPE, 5 × Denhardt's solution, 0.5% SDS (19)) for 1 h at the hybridization temperature (see below). Duplicate membranes were hybridized with each one of a pair of $^{32}$P-end-labeled oligonucleotide probes (13–17 bp; 1 μC/ml), complementary to either the normal sequence or the mutant sequence. Hybridization was for 1 h at a temperature 12°C below the melting temperature of the oligonucleotides; the membranes were then washed briefly three times in washing buffer (5 × SSC, 0.1% SDS) at room temperature and then for 10 min at the stringent temperature, usually 5°C below the melting temperature of the oligonucleotides. In some cases, the stringent wash was at the melting temperature of the oligomers with 0.2 × SSC, 0.1% SDS. Bound oligonucleotides were detected by autoradiography for 2–18 h at -70°C with Omat-XAR5 film and intensifying screens. Control samples of amplified DNA known to be heterozygous for each mutation were always included.

Samples that appeared to be positive for a given mutation by any of the methods described above were checked by direct nucleotide sequencing of a fresh PCR product from the original DNA sample from the patient, and from affected relatives where possible.

### Analysis and nucleotide sequencing of mRNA

Total RNA was isolated from cultured cells that had been preincubated with lipoprotein-deficient serum as previously described (20). The LDL-receptor cDNA was amplified by RT-PCR and the products were digested with restriction enzymes for polymorphic sites as described previously to determine the relative expression of mRNA derived from the two alleles (8, 10). Single-stranded amplified overlapping fragments of LDL-receptor cDNA (approx. 1 kb) for automated nucleotide sequencing were produced by nested RT-PCR of total cytoplasmic RNA, with one of the second-round primers biotinylated. The biotinylated product (approx. 1 μg) was bound to magnetic beads (Dynabeads) and the non-biotinylated strand eluted from the beads after denaturation, following the protocol recommended by the supplier. The single-stranded product attached to the beads was sequenced using the PRISM™ Sequenase Terminator Single-Stranded DNA sequencing kit (Applied Biosystems) with internal primers, as described in the supplier's protocol.

### Measurement of LDL receptor activity in cultured cells

The maximum rate of saturable degradation of $^{125}$I-labeled LDL by cultured skin fibroblasts or cultured Epstein-Barr virus-transformed lymphoblasts was determined as described elsewhere (10, 21).

## RESULTS AND DISCUSSION

### Identification of the mutations in the homozygous patients

The mutations that have now been identified in the LDL-receptor gene of the 15 homozygous FH patients that have been available to us for study are shown in **Table 2.** Seven of these have not been described previously.

In 6 of the 15 patients (patients 10–15 in Table 2), there is evidence of consanguinity in the parents and we have now shown that 5 of these patients are homozygous at the LDL-receptor gene locus. The sixth patient, of Asian Indian origin (patient 10 in Table 2), despite having parents who believe themselves to be first cousins, is heterozygous for the Pro664Leu mutation that has been observed previously in Indian Asians from the same geographical location (20). The index patient has one clinically heterozygous sibling who does not carry the Pro664Leu mutation and one clinically homozygous FH sibling who is also heterozygous for Pro664Leu. In addition, both parents are hypercholesterolemic but



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only the father carries the Pro664Leu mutation, so there is little doubt that there are two different affected alleles in the family, an observation that is supported by haplotype analysis of this gene locus (**Fig. 1**). The finding that the three children and the mother are clearly heterozygous at a number of polymorphic sites rules out a large deletion at the LDL-receptor locus in the maternal allele but, as yet, no mutation in the second allele has been identified by sequencing of the coding region, the immediate intron-exon junctions or 600 bp of the promoter region of the gene in this family. However, analysis of mRNA from cells from the homozygous patient revealed that the mRNA contained almost exclusively the mRNA species for the Pro664Leu allele (see below), so presumably there is a mutation in some region of the gene that has a profound effect on the production or stability of the mRNA.

There is no evidence of consanguinity in the parentage of the 9 patients of apparently either English or part English ancestry (patients 1-9), and this is supported by the observation that they are all compound heterozygotes at the LDL-receptor gene locus. In patients 2 and 4-7, the existence of the two different mutations on separate alleles in the proband was supported by analyzing genomic DNA from affected relatives (**Fig. 2,** (8)). Samples from the parents or other relatives of the remaining compound heterozygous patients were unobtainable, but the point mutations detected in these patients have been observed independently in apparently unrelated heterozygous FH patients, as described below. Apart from the Pro664Leu and Glu80Lys mutations that we have described previously, which each occur in two apparently unrelated individuals, none of the mutations was found in more than one clinically homozygous patient of English ancestry.

The list of mutations in Table 2 includes eight point mutations and a microdeletion that had not been described when our study commenced. The majority of these were detected by direct nucleotide sequence analysis of amplified fragments of genomic DNA isolated either from whole blood or from cultured cells from the patient (**Fig. 3, A-D**). The mutations predicted to cause Arg329Pro in patient 6, Asp461Asn in patient 7, and Cys176Arg in patient 9 were initially detected by automated nucleotide sequencing of mRNA amplified by RT-PCR from cultured lymphoblasts (Fig. 3, G-J), and confirmed by manual sequencing of amplified genomic DNA. After detection of a 21 bp deletion in exon 4 of patient 7 by direct sequencing of the PCR product of genomic DNA, the fragment was cloned and the nucleotide sequence of two normal and two mutant plasmid clones was determined. This confirmed the nature of the deletion and showed that the other allele was normal in the region of the sequence obscured by the deletion (Fig. 3, E).

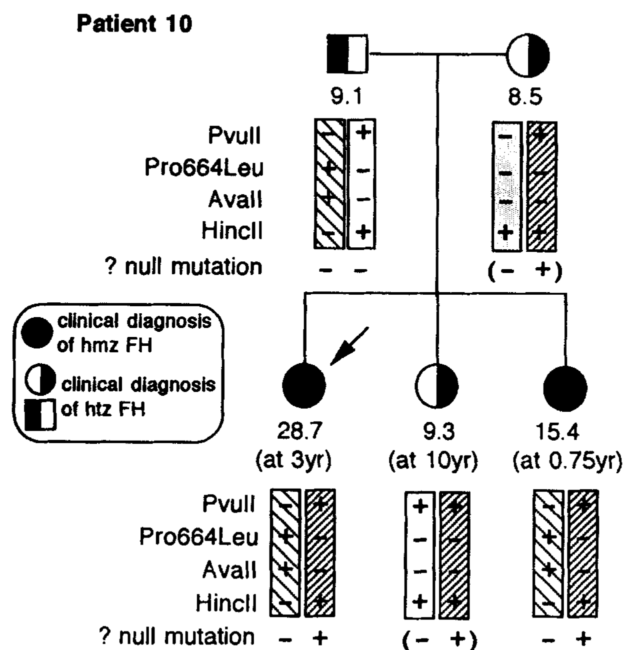


**Fig. 1.** Pedigree of patient 10 showing the haplotypes of the LDL-receptor gene alleles. The clinically homozygous FH proband is indicated by an arrow. The haplotype of each LDL-receptor gene allele, assuming that there has been no recombination, was assigned on the basis of the presence (+) or absence (-) of polymorphic restriction enzyme sites in the gene and on the presence or absence of the Pro664Leu mutation. It was assumed that the clinically heterozygous FH sister of the two probands has inherited from their father the mutant allele that produces an undetectable amount of mRNA in cells from both homozygous siblings (null mutation), as indicated by brackets. The two putative mutant alleles are indicated with hatched boxes and the two normal alleles with plain or shaded boxes. The numbers shown below each symbol indicate the total plasma cholesterol value in mmol/l. Hmz, homozygous; htz, heterozygous.

Two mutant alleles in this group of 15 patients have yet to be characterized, but restriction enzyme digestion of amplified fragments of the LDL-receptor mRNA isolated from cultured cells from the two patients (patients 8 and 10) have shown that although mRNA from the known mutant allele is present, the unknown mutant allele produces no detectable mRNA (**Fig. 4**). Thus it is probable that the unknown genetic defect lies in or near the LDL-receptor gene itself, rather than in a regulatory protein that would affect the expression of both alleles.

Some of the other mutations have been described previously by ourselves in these same patients, for example the large deletions in patients 1 and 2 (7) and the point mutations in patient 4 (8), patient 12 (20) and patient 14 (16). Skin fibroblasts from some of our patients are also present in the "Dallas Collection" (5), and mutations in some of these have probably also been described by Hobbs and co-workers (5) as FH London-5 (Leu578Ser), FH Greece-2 (Ser265Arg), FH Baltimore-1 (Asp283Glu), FH London-4 (Asp69Gly), and FH Cyprus



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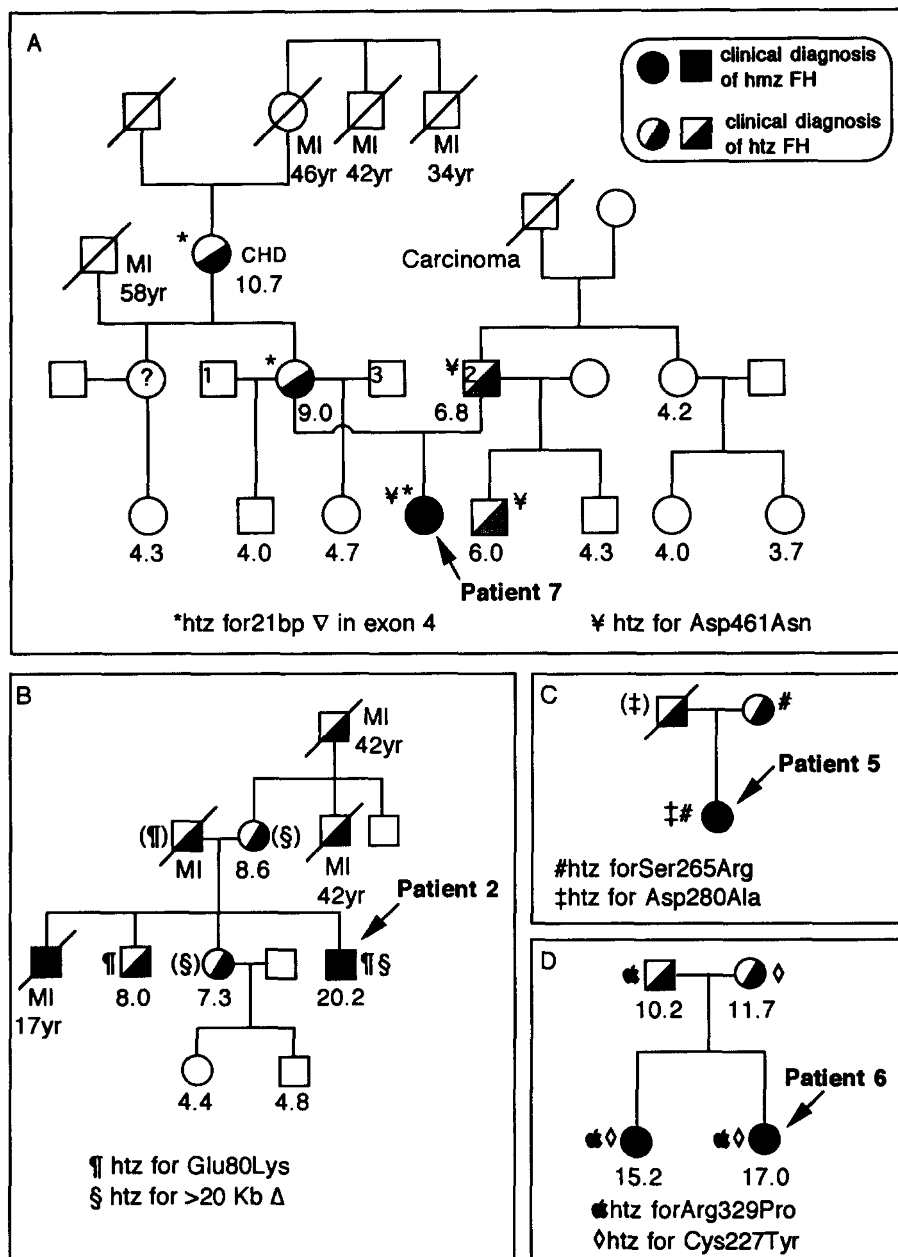


**Fig. 2.** Pedigrees of homozygous FH patients, showing the inheritance of different mutant alleles from each parent. The homozygous (hmz) probands (filled symbols) are indicated by arrows. The numbers below each symbol are the total plasma cholesterol concentration in mmol/l. The half-filled symbols indicate individuals with a clinical diagnosis of heterozygous (htz) FH; the half-shaded symbols in (A) indicate individuals without a clinical diagnosis of FH, but who have a mutation in their LDL-receptor gene that is present in their homozygous relative. The mother of the homozygous proband in (A) has children by three marriages, indicated in order by the numbers within the symbols for the fathers. Where blood samples were not available (B, C), or insufficient DNA was available for Southern blotting to detect the deletion in (B), the presence of a mutation in some of the family members has been deduced, as indicated by brackets round the symbols for the mutations. In (B), the absence of the Glu80Lys mutation from the proband's mother implied that she carried the allele with the deletion and that the father carried the allele with the Glu80Lys mutation. As more than one sibling carried each mutation, it is unlikely that either occurred de novo in the parents.

(Cys292Stop). However, a point mutation in the second allele of our patient carrying the Ser265Arg allele (Asp280Ala) was not reported by these authors.

Perhaps of most interest is the observation that some of the mutations are recurrent. We already have evidence that the Pro664Leu mutation is recurrent (6), and the frequency with which we have found this mutation in additional patients of diverse ethnic origin provides



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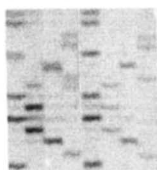
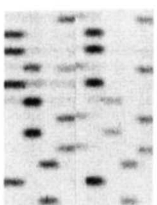
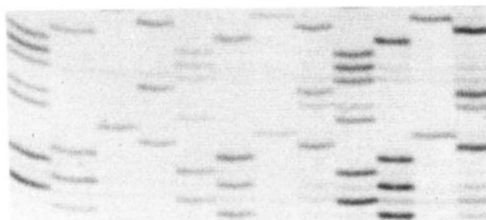
A. **Patient 5**, Exon 6.



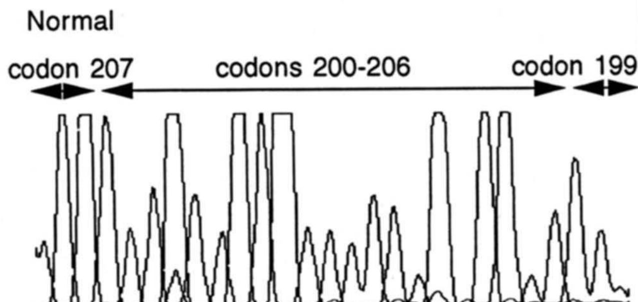



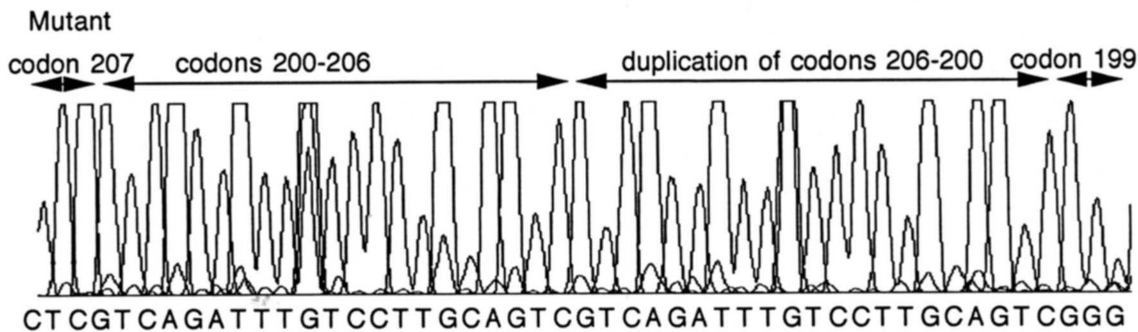

B. **Patient 15**, Exon 6




D. **Patient 13**, Exon 9

E. **Patient 7**, Exon 4 (non-coding strand)




C. **Patient 3**, Exon 6

further confirmation of at least two mutational events. The 21 bp insertion in exon 4 is also likely to have recurred, as Hobbs and coworkers (5) have apparently identified this mutation in an African American and it is unlikely that the two individuals share a common ancestor. Similarly, we have also observed the Glu387Lys mutation in a heterozygous patient of Welsh origin, who is unlikely to share a common ancestor with



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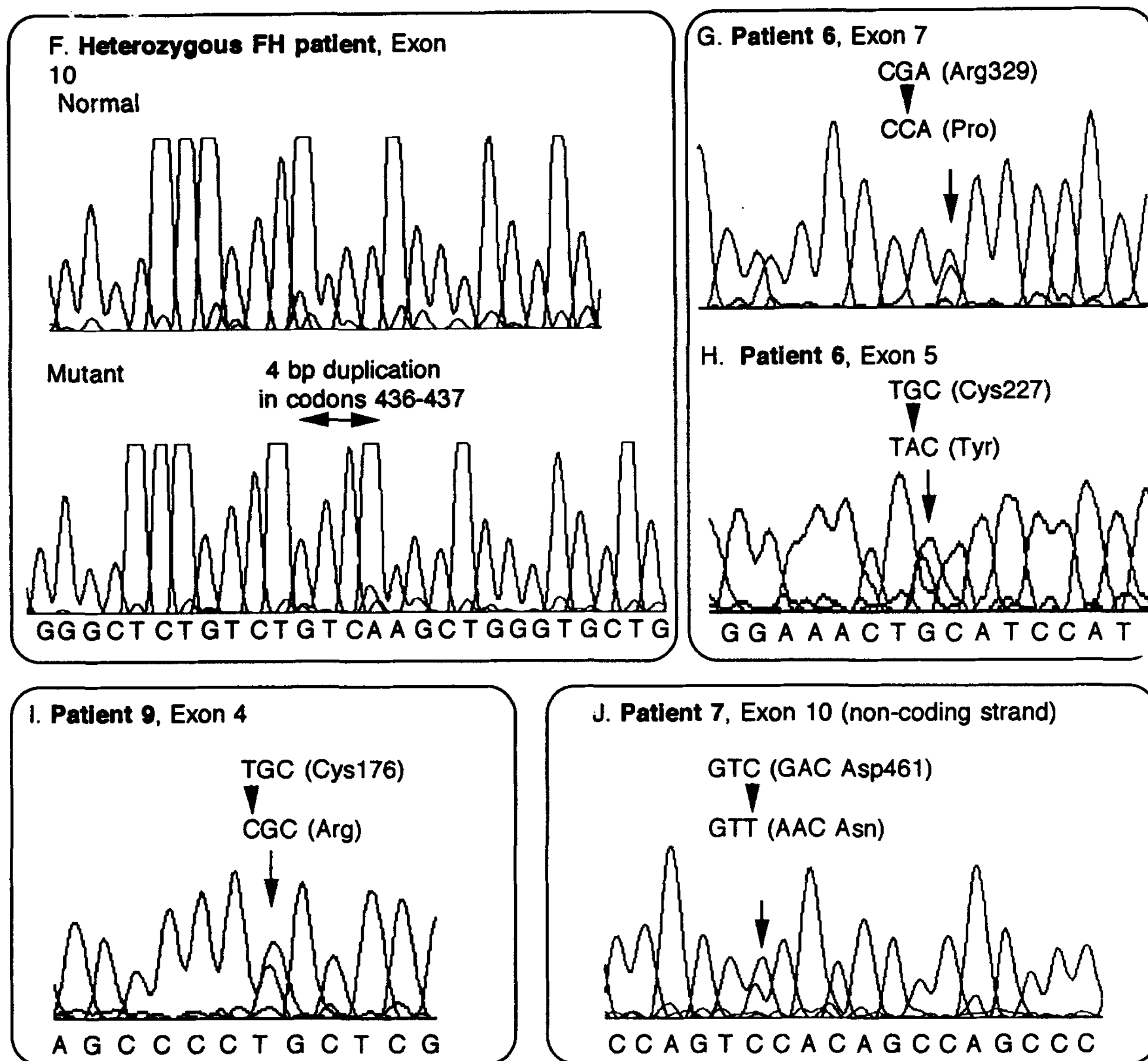


**Fig. 3.** Nucleotide sequence of normal and mutant fragments of the LDL-receptor gene or its mRNA. A–D: Direct manual sequencing of amplified fragments of genomic DNA. Exons 5 + 6 and exons 9 + 10 were amplified with primers located at the appropriate intron/exon junctions (5′-ttagtcgacCACACTCTGTCCTGTTTTCCA and 5′-atagtcgacGCAAGCCGCCTGCACCGAGAC for exons 5 + 6 and 5′-aaagtcgacCTGACCTCGCTCCCCGGGACC (primer 537) and 5′-aaagtcgacGCCCTCAGCGTCGTGGATACG for exons 9 + 10; lower case letters indicate non-LDL-receptor-gene sequences containing a restriction enzyme site. Exon 6 was sequenced with a primer at the 3′ end of intron 5 (5′-TCCTTCCTCTCTCTGGCT); exon 9 was sequenced with primer 537. E and F: Automated plasmid cycle sequencing of cloned fragments of amplified genomic DNA. The 3′ end of exon 4 was amplified with a primer in exon 4 (5′-CCCCAGCTGTGGGCCTGCGACAA) and primer 571, located at the junction of exon 4 and intron 4 (5′-atagtcgacCGCCCCGCCCCCACCCTGCCC); exons 9 + 10 were amplified together as described above. The products were cloned into pGem-T and sequenced with a primer in the vector for exon 10 (T7 promoter, 5′-TAATACGACTCACTATAGGG) and primer 571 for exon 4. G–J: Automated sequencing of RT-PCR-amplified fragments of mRNA with Sequenase. Exons 7 (G) and 10 (J) were contained in a biotinylated PCR product that comprised nucleotides 846 to 1778, amplified with the following primers: biotinyl-5′-CAAGTGTCACAGCGGCGAATG and 5′-CCCCCATTGACATCGATGCTT, and sequenced with a primer encompassing nucleotides 1331 to 1301 for exon 7 and 1685 to 1705 for exon 10. The fragment containing exons 4 (I) and 5 (H) comprised nucleotides 349 to 901 and was amplified with biotinylated 5′-CTCTAGCCATGTTGCAGACTT and 5′-CACGATGGGAAGTGCATCTCTC and sequenced with a primer encompassing nucleotides 413–434.

the homozygous patient of Indian Asian origin. Four other point mutations, Cys176Arg, Cys227Tyr, Asp283Glu, and Arg329Pro, occur in codons in which other mutations have been described (5, 22).

### Effect of the mutations on LDL-receptor activity

There is little doubt that the point mutations described here for the first time are the underlying cause of FH in these patients. All of them are predicted to



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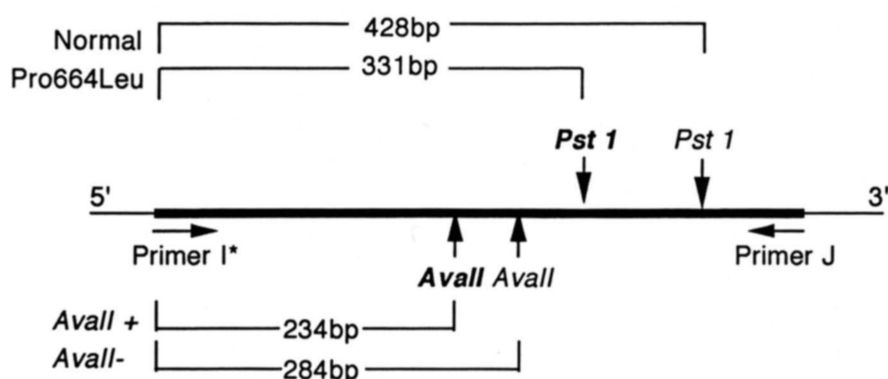


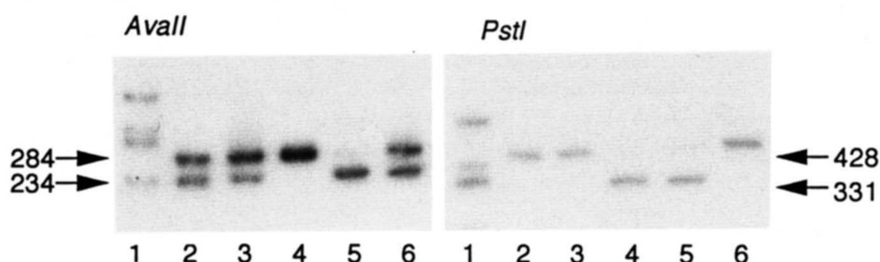


**Fig. 4.** Analysis of mRNA from compound heterozygous FH patients. Total cytoplasmic RNA was isolated from cultured lymphoblasts or skin fibroblasts that had been incubated for 12 h in lipoprotein deficient serum to induce expression of the LDL-receptor gene. (a) A 500 bp fragment of the mRNA was amplified by RT-PCR with the following primers: 5′-CCTCTACTGGGTTGACTCCAA, end-labeled with $^{32}P$ (I*, in exon 12) and 5′-GAGGTGTCGGGAACAGGCCGG (J, in exon 14), as shown in the diagram. The polymorphic *Ava*II site in exon 13 and the *Pst*I site introduced by the Pro664Leu mutation in exon 14 are indicated in bold type. The sizes of the labeled fragments obtained after digestion with either enzyme are indicated. (b) The labeled PCR products were digested with either *Ava*II (left panel) or *Pst*I (right panel) and analyzed by agarose gel electrophoresis followed by autoradiography. Lane 1, markers; lanes 2 and 3, mRNA from lymphoblasts from normolipemic controls; lane 4, mRNA from fibroblasts from patient 8; lane 5, mRNA from fibroblasts from patient 10; lane 6, mRNA from lymphoblasts from patient 7. All three patients are heterozygous in genomic DNA for the *Ava*II RFLP and patients 8 and 10 are heterozygous for the Pro664Leu mutation that creates a *Pst*I site. Patient 7 has mutations in exon 4 and exon 10 (see Table 2). The arrows on the left indicate the sizes of the predicted fragments (bp).

result in non-conservative amino acid substitutions, several involving cysteine residues, in regions of the LDL-receptor protein whose structure and function are now well established to be sensitive to such changes from studies of the expression of mutant forms of the human LDL receptor in heterologous cells (4, 8, 23). In addition, with the exception of the Asp280Ala mutation, all the new point mutations reported in Table 2 have either been observed in an affected relative of the patient or have been found in other unrelated heterozygous FH patients, as described below.

Residual LDL-receptor activity in cells from all but two of the homozygous patients is shown in Table 2. Patients 7, 11, 13, and 14 have very low or virtually undetectable LDL-receptor activity in their cells, which is consistent with the nature of the genetic defect and with the absence of immunodetectable LDL-receptor protein in cell extracts (data not shown). Another group, including patients 1, 2, 5, 8, and 13 have low but detectable activity, while a third group have at least 20% of the LDL-receptor activity detected in cells from normolipaemic controls (patients 3, 4, 9, and 12). As shown in **Fig. 5** and described below, there was no correlation between residual LDL-receptor activity in cultured cells from the



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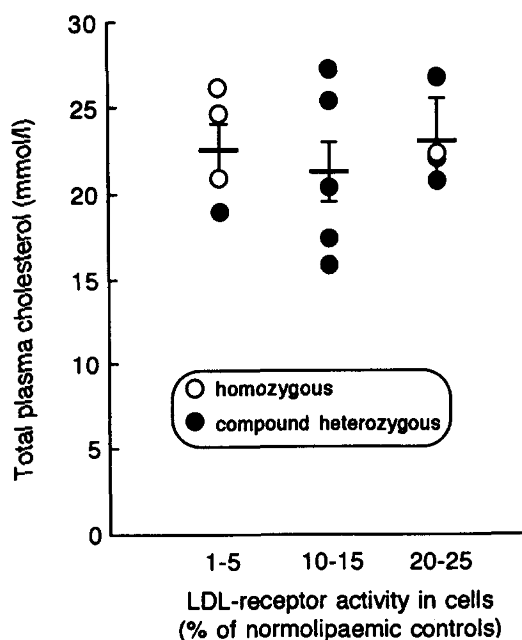


**Fig. 5.** Relationship between plasma cholesterol concentration and LDL-receptor activity in cultured cells from homozygous FH patients. The maximum rate of saturable degradation of $^{125}$I-labeled LDL by cultured skin fibroblasts or EBV-transformed lymphoblasts was expressed as a percentage of the values obtained with cells of the same type from normolipemic individuals during the same experiment. The horizontal lines show the mean value in each group ± SEM.

patients and their plasma cholesterol concentration before treatment commenced. However, some caution is necessary in interpreting these results because assays of LDL-receptor activity in cultured cells preincubated with lipoprotein-deficient serum in vitro may not be a completely accurate reflection of LDL-receptor function in vivo. Furthermore, considerable variation in measurable LDL-receptor activity is found in cells from normolipemic controls, both between individuals and even in cells from the same individual, depending on cell density.

**Effect of mutations on clinical phenotype**

The mean age of the seven male and eight female homozygotes was 24.4 years, ranging from 7 to 45 (Table 1). Their untreated serum cholesterol levels averaged 23.1 mmol/l with a range of 15.6–30.6 mmol/l. Atherosclerotic involvement of the aortic root and valve, together with the coronary and/or carotid arteries, was documented in all those aged 17 or above. In four instances this had proved fatal despite surgical intervention and apheresis. LDL apheresis has been shown to prolong survival (24) but should be commenced well before puberty if aortic valve stenosis is to be prevented. Aortic valve replacement in homozygotes is a risky procedure and led to two post-operative deaths (Cases 3 and 8). However, it is noteworthy that the oldest patient in our series, case 5, had her aortic valve replaced 16 years ago (24).

The age of onset of aorto-coronary atherosclerosis is closely correlated with the age and mean serum cholesterol throughout life of the individual concerned (25). We do not have sufficient data on the combined effects of non-pharmacological and pharmacological treatment on serum cholesterol levels in these patients to be able to calculate the age × cholesterol score in every instance. However, the age of onset and frequency of cardiovascular involvement in our patients was similar to that reported by Hoeg, Feuerstein, and Tucker (25). Although variability in the severity of hypercholesterolemia in homozygotes has been regarded in the past as being determined by the effects of the underlying mutation(s) on LDL receptor expression (2, 26), as shown in Fig. 5 there was no correlation between untreated serum cholesterol levels and residual LDL receptor activity in cultured cells in our patients.

The majority of the mutations found in the homozygous FH patients are associated with severe hypercholesterolemia in their affected heterozygous relatives. The one exception is the mutation predicted to cause substitution of Asp461 with Asn in exon 10 of the gene, which codes for part of the region with homology to the epidermal growth factor precursor (27). The homozygous child, patient 7, inherited this allele from her father, who has also passed it on to one of his two sons by a subsequent marriage (Fig. 2). The father's total plasma cholesterol concentration was 6.8 mmol/l and his LDL-cholesterol concentration was 4.9 mmol/l, and the son's values for these parameters were even lower (6.1 and 4.3 mmol/l, respectively). Thus, these individuals would not normally be given a diagnosis of heterozygous FH, as neither have detectable tendon xanthoma, corneal arcus, clinical signs of CHD or a family history of CHD. It might be expected that this mutation, which has a relatively mild effect in her heterozygous parent and half-brother, would not result in the typically severe phenotype seen in patient 7, who showed all the features of homozygous FH at an early age (Table 1). We considered the possibility that the second defective allele, with the 21 bp duplication, interferes with expression of the Asp461Asn allele, but analysis of the mRNA from cultured lymphoblasts from patient 7 showed that mRNA from both alleles was present in the cells in approximately equal proportions (Fig. 4). However, we could not determine unequivocally whether both mRNA species were reduced in concentration compared with cells from a normolipiaemic individual.

Two other heterozygous carriers of this mutant allele (Asp461Asn) have been identified in two groups of FH patients screened for mutations (see below). One of



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TABLE 3. Identification of carriers of mutations detected in the LDL-receptor gene in the UK in two groups of patients with a diagnosis of heterozygous FH

| Mutation[a] | Location | Report of Original Patient with Mutation | Detection Method | Number of Patients with Mutation | |
|---|---|---|---|---|---|
| | | | | 200 FH[a] | 95 HH[b] |
| G + 1A | Intron 3 | (10) | Hybridization with ASO | 2[c] | 0 |
| Glu80Lys | Exon 3 | (8) | Hybridization with ASO | 6[c] | 0 |
| Asp69Gly | Exon 3 | Hmz FH in this study | Hybridization with ASO | 3[c] | 2 |
| Trp66Gly | Exon 3 | (34) | Hybridization with ASO | 0 | 2 |
| Cys88Tyr | Exon 4 | (31) | New *Rsa*I site, PAGE | 1 | 0 |
| Cys176Arg | Exon 4 | Hmz FH in this study | Hybridization with ASO | 0 | 1 |
| Δ Gly197 | Exon 4 | (35) | Heteroduplexes on PAGE | 6[e] | 2 |
| Dup. codons 200–206 | Exon 4 | Hmz FH in this study | Heteroduplexes on PAGE | 1[d] | 0 |
| Δ 2b in codon 206 | Exon 4 | (9) | Heteroduplexes on PAGE | 5[e] | 1 |
| Asp206Glu | Exon 4 | (36) | New *Dde*I site, PAGE | 3[e] | 0 |
| Cys227Tyr | Exon 5 | Hmz FH in this study | Hybridization with ASO | 1 | 0 |
| Asp280Ala | Exon 6 | Hmz FH in this study | Destroys *Bsm*AI site, agarose gel | 1[d] | 0 |
| Asp283Glu | Exon 6 | Hmz FH in this study | Hybridization with ASO | 1[d] | 0 |
| Arg329Pro | Exon 7 | Hmz FH in this study | Hybridization with ASO | 2[d] | 0 |
| Glu387Lys | Exon 9 | Hmz FH in this study | Hybridization with ASO | 1[d] | 0 |
| Deletion/insertion | Intron 9 | Webb & Soutar, unpublished | Heteroduplexes on PAGE | 1 | 0 |
| Dup. 4 bp | Exon 10 | Htz FH in this study | Heteroduplexes on PAGE | 2 | 0 |
| Asp461Asn | Exon 10 | Hmz FH in this study | Hybridization with ASO | 1 | 1 |
| Leu578Ser | Exon 12 | Hmz FH in this study | Hybridization with ASO | 1[d] | 1 |
| Arg612Cys | Exon 12 | (32) | Hybridization with ASO | 2[d] | 1 |
| Pro664Leu | Exon 14 | (20) | New *Pst*I site, agarose gel | 6 | 1 |

Abbreviations: Δ deletion; dup, duplication; Hmz, homozygous; ASO, allele specific oligonucleotides; Htz, heterozygous; PAGE, polyacrylamide gel electrophoresis.
[a]Two hundred unrelated FH patients attending one of several Lipid Clinics in the London area (7).
[b]Ninety five unrelated FH patients attending Hammersmith Hospital Lipid Clinic.
[c]Data from ref. 10.
[d]Number includes the homozygous patient in whom the mutation was detected (Table 1).
[e]Data from ref. 9.

these, with a diagnosis of possible FH, was referred after a routine health check had identified a raised plasma cholesterol of 10.0 mmol/l with an LDL-cholesterol of 8.15 mmol/l, but he has no tendon xanthoma and no detectable CHD at the age of 54 years. This patient's father died of CHD at the age of 66 years. The second individual is a 47-year-old female with a pre-treatment plasma cholesterol of 8.3 mmol/l that fell to between 5.9 and 7.2 mmol/l on treatment with diet alone, but in contrast to the other patients with this mutation, she already had tendon xanthoma and carotid artery disease at the age of 31 years. She has a strong maternal and paternal family history of premature CHD, suggesting that she may have more than one inherited risk factor for early atherosclerosis. Even though the number of patients with this mutation is small, it is of interest that adult carriers of the same mutant allele can exhibit pre-treatment plasma cholesterol concentrations that range from 6.8 to 10.0 mmol/l and a wide range of susceptibility to atherosclerosis that is apparently unrelated to the plasma cholesterol concentration, confirming that other factors must have an important influence. Presumably, patients with heterozygous FH are also subject to the same polygenic and environmental factors that influence CHD risk in the apparently normolipemic population. These might include, for example, diet, smoking, and obesity, and variants of the many genes involved in lipid and lipoprotein metabolism, the clotting mechanism or cell biology of the arterial wall. Studies of Chinese (4) and Tunisian (28) FH patients show that where the mean plasma cholesterol concentration of the general population is low, then heterozygous FH patients are much less severely affected, if at all, than those in Western societies where the mean plasma cholesterol concentration is higher. The simplest explanation for these differences is that they are caused by differences in diet, but unrelated genetic factors such as the dominant gene effect observed in a Puerto Rican family (29) cannot be ruled out.

### Screening for the mutations in heterozygous FH patients

Although we had predicted that the mutations that occurred in the clinically homozygous patients would be likely to be relatively common amongst heterozygous FH patients attending Lipid Clinics in the London area, this did not turn out to be the case when genomic DNA from 295 individuals was screened for the mutations that we had identified (**Table 3**). The homozygous patients, with the exception of numbers 4, 7, and 9 in Table



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2, were included in the group of 200 patients shown in Table 3.

Where the mutation introduced or destroyed a restriction enzyme site, amplified DNA from the patients was digested with the enzyme and the products were analyzed by either agarose gel electrophoresis or non-denaturing PAGE. Mutations that formed detectable heteroduplexes were analyzed by non-denaturing PAGE of the amplified fragment. Screening for all other point mutations was carried out by hybridization of the amplified fragment with allele-specific oligonucleotides. As indicated in Table 3, in addition to 14 different point mutations, micro-deletions, -insertions, and -rearrangements found in the 9 homozygous patients of apparently English ancestry, we also screened for 10 mutations of this type that we have identified in single individuals in a separate group of heterozygous FH patients in another study (10, 30, 31) and for a point mutation (Arg612Cys) that we identified in a further homozygous patient who was not in our cohort (32). The new group of 95 patients were also screened for three of the commoner mutations in exon 4 (9). Out of the total number of 29 mutations tested, the following were not observed in any individuals in the groups of 200 or 95 FH patients: Ala585Ser, a single bp insertion in exon 5, a 23 bp deletion in exon 7; a 4 bp deletion in exon 10, a single bp deletion in exon 13, a single bp deletion in exon 14 and a mutation in the promoter region. The single bp deletion in exon 15 identified in one of the homozygous patients (patient 4) who was not in the group of 200 was not seen in any additional patients.

During screening for microdeletions by non-denaturing PAGE of an amplified fragment comprising exons 9 and 10, heteroduplexes were detected in three samples. Nucleotide sequencing of the fragments revealed these to be the same duplication of 4 bp in codons 436 and 437 in two patients (Fig. 3, F), and a complex insertion/deletion at the 3′ end of intron 9 that disrupts splicing in the third patient (J. C. Webb and A. K. Soutar, unpublished observations).

As shown in Table 3, the majority of the new mutations were only found in the index patient and were not even present at the frequency of approximately 5% that we have observed previously for a number of other mutations, namely Glu80Lys, Pro664Leu, and two microdeletions in exon 4 (6, 8, 9). A summary of all the mutations identified so far in the group of 200 FH patients is shown in **Table 4.** The mutations described in this paper increase the total number of alleles characterized in this group from 38 to 71, that is from 18% to 34% of the predicted total number of 208 defective LDL-receptor gene alleles. Thus, potentially 137 mutations remain unidentified, but current studies elsewhere using large scale SSCP analysis (33) should identify some of these. In the new group of 95 patients, only 12 alleles out of a total of 98 (12%) have been identified by our screening procedure, but this group has not been analyzed for large deletions or rearrangements that comprise approximately 5% of the mutant alleles in the group of 200 (7), nor for two of the rarer mutations in exon 4 described by Gudnason and coworkers (9) that together comprise about 1% in the group of 200 FH patients. Furthermore, the group of 200 contains the majority of the homozygous patients in whom we actively sought mutations.

TABLE 4. Summary of mutations identified in 200 FH patients attending Lipid Clinics in the London area

| Mutation | Number of Patients | Reference |
|---|---|---|
| Pro664Leu | 6 | (6) |
| Deletions detected by Southern blotting | 10[a] | (7) |
| Glu80Lys | 6 | (8) |
| Minor base changes in exon 4 | 21 | (9) |
| G + 1A in intron 3 | 2 | (10) |
| Screening for known point mutations | 17 | this paper |
| Cys292Stop | 2[b] | this paper |
| Asp471Asn | 1[c] | this paper |
| Total alleles[d] (percentage) | 71 (34.3%) | |

[a]One additional patient with a deletion has been detected since this publication.
[b]Includes a Greek Cypriot homozygous (see Table 2) and one Greek heterozygous FH patient; non-Greek remainder of 200 not screened.
[c]One heterozygous FH patient of Chinese origin; non-Chinese remainder of 200 not screened.
[d]The 200 patients include 189 heterozygous and 11 homozygous FH, of which 8 are compound heterozygous, giving a total of 208 potentially affected alleles.

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## REFERENCES

1. Goldstein, J. L., and M. S. Brown. 1995. Familial hypercholesterolemia. *In* The Metabolic Basis of Inherited Disease. Vol. II. 7th ed. C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle, editors. McGraw-Hill, New York. 1215–1250.
2. Moorjani, S., M. Roy, A. Torres, C. Betard, C. Gagne, M. Lambert, D. Brun, J. Davignon, and P. Lupien. 1993.



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Mutations of low-density-lipoprotein-receptor gene, variation in plasma cholesterol, and expression of coronary heart disease in homozygous familial hypercholesterolaemia. *Lancet.* **341:** 1303–1306.

3. Hobbs, H. H., D. W. Russell, M. S. Brown, and J. L. Goldstein. 1990. The LDL receptor locus in familial hypercholesterolemia: mutational analysis of a membrane protein. *Annu. Rev. Genet.* **24:** 133–170.
4. Sun, X-M., D. D. Patel, J. C. Webb, B. L. Knight, L-M. Fan, H-J. Cai, and A. K. Soutar. 1994. Familial hypercholesterolemia in China: identification of mutations in the LDL receptor gene that result in a receptor negative phenotype. *Arterioscler. Thromb.* **14:** 85–94.
5. Hobbs, H. H., M. S. Brown, and J. L. Goldstein. 1992. Molecular genetics of the LDL receptor gene in familial hypercholesterolemia. *Hum. Mutat.* **1:** 445–466.
6. King, U. L., V. Gudnason, S. Humphries, M. Seed, D. Patel, B. Knight, and A. Soutar. 1991. Identification of the 664 proline to leucine mutation in the low density lipoprotein receptor in four unrelated patients with familial hypercholesterolaemia in the UK. *Clin. Genet.* **40:** 17–28.
7. Sun, X. M., J. C. Webb, V. Gudnason, S. Humphries, M. Seed, G. R. Thompson, B. L. Knight, and A. K. Soutar. 1992. Characterization of deletions in the LDL receptor gene in patients with familial hypercholesterolemia in the United Kingdom. *Arterioscler. Thromb.* **12:** 762–770.
8. Webb, J. C., X. M. Sun, D. D. Patel, S. N. McCarthy, B. L. Knight, and A. K. Soutar. 1992. Characterization of two new point mutations in the low density lipoprotein receptor genes of an English patient with homozygous familial hypercholesterolemia. *J. Lipid Res.* **33:** 689–698.
9. Gudnason, V., U. L. King, M. Seed, X. M. Sun, A. K. Soutar, and S. E. Humphries. 1993. Identification of recurrent and novel mutations in exon 4 of the LDL receptor gene in patients with familial hypercholesterolemia in the United Kingdom. *Arterioscler. Thromb.* **13:** 56–63.
10. Sun, X. M., D. D. Patel, D. Bhatnagar, B. L. Knight, and A. K. Soutar. 1995. Characterization of a splice-site mutation in the gene for the LDL receptor associated with an unpredictably severe clinical phenotype in English patients with heterozygous FH. *Arterioscler. Thromb. Vasc. Biol.* **15:** 219–227.
11. Barbir, M., A. Khaghani, A. Kehely, K. C. Tan, A. Mitchell, G. R. Thompson, and M. Yacoub. 1992. Normal levels of lipoproteins including lipoprotein(a) after liver–heart transplantation in a patient with homozygous familial hypercholesterolaemia. *Q. J. Med.* **85:** 807–812.
12. Allen, J. M., G. R. Thompson, N. B. Myant, R. Steiner, and C. M. Oakley. 1980. Cardiovascular complications of homozygous familial hypercholesterolaemia. *Br. Heart J.* **44:** 361–368.
13. Hendry, W. G., and M. Seed. 1985. Homozygous familial hypercholesterolaemia with supravalvar aortic stenosis treated by surgery. *J. R. Soc. Med.* **78:** 334–335.
14. Kitano, Y., and G. R. Thompson. 1993. Role of LDL apheresis in the management of hypercholesterolaemia. *Transfus. Sci.* **14:** 269–280.
15. West, R., P. Gibson, and J. Lloyd. 1985. Treatment of homozygous familial hypercholesterolaemia: an informative sibship. *Br. Med. J. Clin. Res. Ed.* **291:** 1079–1080.
16. Feher, M. D., J. C. Webb, D. D. Patel, A. F. Lant, P. D. Mayne, B. L. Knight, and A. K. Soutar. 1993. Cholesterol-lowering drug therapy in a patient with receptor-negative homozygous familial hypercholesterolaemia. *Atherosclerosis.* **103:** 171–180.
17. Scientific Steering Committee on behalf of the Simon Broom Register Group, 1991. Risk of fatal coronary heart disease in familial hypercholesterolaemia. *Br. Med. J.* **303:** 893–896.
18. Tybjaerg-Hansen, A., J. Gallagher, J. Vincent, R. Houlston, P. Talmud, A. M. Dunning, M. Seed, A. Hamsten, S. E. Humphries, and N. B. Myant. 1990. Familial defective apolipoprotein B-100: detection in the United Kingdom and Scandinavia, and clinical characteristics of ten cases. *Atherosclerosis.* **80:** 235–242.
19. Maniatis, T. 1989. Analysis of genomic DNA by Southern hybridization. *In* Molecular Cloning: A Laboratory Manual. 2nd ed. J. Sambrook, E. F. Fritsch, and T. Maniatis, editors. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. 9.31–9.57.
20. Soutar, A. K., B. L. Knight, and D. D. Patel. 1989. Identification of a point mutation in growth factor repeat C of the low density lipoprotein-receptor gene in a patient with homozygous familial hypercholesterolemia that affects ligand binding and intracellular movement of receptors. *Proc. Natl. Acad. Sci. USA.* **86:** 4166–4170.
21. Goldstein, J. L., and M. S. Brown. 1974. Binding and degradation of low density lipoproteins by cultured human fibroblasts. Comparison of cells from a normal subject and from a patient with homozygous familial hypercholesterolemia. *J. Biol. Chem.* **249:** 5153–5162.
22. Solberg, K., O. K. Rodningen, S. Tonstad, L. Ose, and T. P. Leren. 1994. Familial hypercholesterolaemia caused by a non-sense mutation in codon 329 of the LDL receptor gene. *Scand. J. Clin. Lab. Invest.* **54:** 605–609.
23. Esser, V., L. E. Limbird, M. S. Brown, J. L. Goldstein, and D. W. Russell. 1988. Mutational analysis of the ligand binding domain of the low density lipoprotein receptor. *J. Biol. Chem.* **263:** 13282–13290.
24. Thompson, G. R., J. P. Miller, and J. L. Breslow. 1985. Improved survival of patients with homozygous familial hypercholesterolaemia treated by plasma exchange. *Br. Med. J.* **291:** 1671–1673.
25. Hoeg, J. M., I. M. Feuerstein, and E. E. Tucker. 1994. Detection and quantitation of calcific atherosclerosis by ultrafast computed tomography in children and young adults with homozygous familial hypercholesterolemia. *Arterioscler. Thromb.* **14:** 1066–1074.
26. Sprecher, D. L., J. M. Hoeg, E. J. Schaefer, L. A. Zech, R. E. Gregg, E. Lakatos, and H. B. Brewer, Jr. 1985. The association of LDL receptor activity, LDL cholesterol level, and clinical course in homozygous familial hypercholesterolemia. *Metabolism.* **34:** 294–299.
27. Sudhof, T. C., J. L. Goldstein, M. S. Brown, and D. W. Russell. 1985. The LDL receptor gene: a mosaic of exons shared with different proteins. *Science.* **228:** 815–822.
28. Slimane, M. N., H. Pousse, F. Maatoug, M. Hammami, and M. H. Ben Farhat. 1993. Phenotypic expression of familial hypercholesterolemia in central and southern Tunisia. *Atherosclerosis.* **104:** 153–158.
29. Hobbs, H. H., E. Leitersdorf, C. C. Leffert, D. R. Cryer, M. S. Brown, and J. L. Goldstein. 1989. Evidence for a dominant gene that suppresses hypercholesterolemia in a family with defective low density lipoprotein receptors. *J. Clin. Invest.* **84:** 656–664.
30. Sun, X-M., C. Neuwirth, D. P. Wade, B. L. Knight, and A. K. Soutar. 1995. A mutation (T-45C) in the promoter region of the low-density-lipoprotein (LDL) receptor gene is associated with a mild clinical phenotype in a patient



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with heterozygous familial hypercholesterolaemia. *Hum. Mol. Genet.* **4:** 2125–2129.
31. Sun, X-M., C. Neuwirth, D. D. Patel, B. L. Knight, and A. K. Soutar. 1995. Phenotypic variation and its correlation with mutations in the low density lipoprotein receptor gene in familial hypercholesterolaemia. *Z. Gastroenterol.* **Suppl 3.** In press.
32. Gudnason, V., D. P. R. Muller, J. K. Lloyd, and S. E. Humphries. 1995. Response to drugs and diet in a compound heterozygote for familial hypercholesterolaemia (FH). *Arch. Dis. Child.* **73:** 538–540.
33. Whittall, R., V. Gudnason, G. P. Weavind, L. B. Day, S. E. Humphries, and I. N. M. Day. 1995. Utilities for high throughput use of the single strand conformational polymorphism method: screening of 791 patients with familial hypercholesterolaemia for mutations in exon 3 of the low density lipoprotein receptor gene. *J. Med. Genet.* **32:** 509–515.
34. Leitersdorf, E., E. J. Tobin, J. Davignon, and H. H. Hobbs. 1990. Common low-density lipoprotein receptor mutations in the French Canadian population. *J. Clin. Invest.* **85:** 1014–1023.
35. Meiner, V., D. Landsberger, N. Berkman, A. Reshef, P. Segal, H. C. Seftel, D. van der Westhuyzen, M. S. Jeenah, G. A. Coetzee, and E. Leitersdorf. 1991. A common Lithuanian mutation causing familial hypercholesterolemia in Ashkenazi Jews. *Am. J. Hum. Genet.* **49:** 443–449.
36. Leitersdorf, E., D. van der Westhuyzen, G. A. Coetzee, and H. H. Hobbs. 1989. Two common low density lipoprotein receptor gene mutations cause familial hypercholesterolemia in Afrikaners. *J. Clin. Invest.* **84:** 954–961.



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