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Mechanisms of Disease: genetic causes of familial hypercholesterolemia

Anne K Soutar* and Rossi P Naoumova

SUMMARY

Familial hypercholesterolemia (FH) is characterized by raised serum LDL cholesterol levels, which result in excess deposition of cholesterol in tissues, leading to accelerated atherosclerosis and increased risk of premature coronary heart disease. FH results from defects in the hepatic uptake and degradation of LDL via the LDL-receptor pathway, commonly caused by a loss-of-function mutation in the LDL-receptor gene (*LDLR*) or by a mutation in the gene encoding apolipoprotein B (*APOB*). FH is primarily an autosomal dominant disorder with a gene–dosage effect. An autosomal recessive form of FH caused by loss-of-function mutations in *LDLRAP1*, which encodes a protein required for clathrin-mediated internalization of the LDL receptor by liver cells, has also been documented. The most recent addition to the database of genes in which defects cause FH is one encoding a member of the proprotein convertase family, *PCSK9*. Rare dominant gain-of-function mutations in *PCSK9* cosegregate with hypercholesterolemia, and one mutation is associated with a particularly severe FH phenotype. Expression of PCSK9 normally downregulates the LDL-receptor pathway by indirectly causing degradation of LDL-receptor protein, and loss-of-function mutations in *PCSK9* result in low plasma LDL levels. Thus, PCSK9 is an attractive target for new drugs aimed at lowering serum LDL cholesterol, which should have additive lipid-lowering effects to the statins currently used.

KEYWORDS apolipoprotein B, coronary heart disease, LDL receptor, *LDLRAP1, PCSK9*

REVIEW CRITERIA

Potentially relevant papers, regardless of publication date, were identified by searching PubMed using the terms "familial hypercholesterol*", "low density lipoprotein (LDL) receptor gene", "familial defective apoB", "autosomal recessive" or "PCSK9" during August 2006. The final reference list is by no means comprehensive and those articles included were selected to illustrate specific points or are reviews containing additional references. All papers quoted are full-text English-language papers.

CME

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Learning objectives

Upon completion of this activity, participants should be able to:

1 Identify the main physiologic defect promoting increased LDL levels in patients with familial hypercholesterolemia.
2 Describe genetic factors that promote familial hypercholesterolemias.
3 Correlate clinical factors with genetic disorders associated with familial hypercholesterolemia.

INTRODUCTION

Familial hypercholesterolemia (FH) is an inherited disorder characterized by a high concentration of serum LDL cholesterol. The high LDL cholesterol level frequently gives rise to xanthomas, deposits of cholesterol in peripheral tissues, and accelerated atherosclerosis resulting from cholesterol deposition in the arterial wall, thereby increasing the risk of premature coronary heart disease (CHD).[1] The precise mode of inheritance was difficult to establish in regions where noninherited hypercholesterolemia was common, and was first defined by Khachadurian in 1964 in Lebanese FH pedigrees.[2] Khachadurian showed that individuals from affected families could be segregated into three clear groups on the basis of their plasma cholesterol concentrations: presumed homozygotes with levels four times higher than normal; heterozygotes with levels two times higher that normal; and unaffected individuals. He concluded that FH was inherited as a monogenic autosomal codominant trait—a dominant disorder with a gene–dosage effect. The frequency of

heterozygous FH in most populations is about 1/500,[1] so homozygous FH is rare (≤1/10^6) in European populations with a low rate of consanguineous marriage, but the pattern of inheritance observed by Khachadurian can also be seen in small families of this origin (Figure 1).

This Review will describe the way in which the genetic basis of FH first came to light, leading to the elucidation of the LDL-receptor pathway, and how defects in different genes were later found to result in the same clinical phenotype. The implications of knowing the genetic defect for clinical management of FH will be discussed. A detailed description of the symptoms and clinical management of FH is outside the remit of this article and has been amply covered elsewhere.[1,3–5]

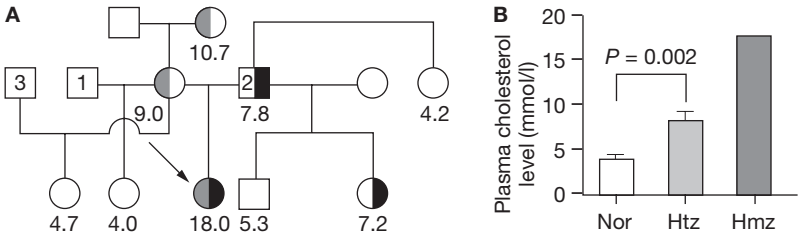

Figure 1 Dominant pattern of inheritance of familial hypercholesterolemia due to mutations in the LDL-receptor gene (*LDLR*). (**A**) The index patient in this family (arrow) is a child with a clinical phenotype of homozygous familial hypercholesterolemia.[29] The dark half-filled symbols indicate individuals heterozygous for the Asp461Asn mutation in *LDLR*; the light half-filled symbols indicate individuals heterozygous for 21-base-pair duplication in exon 4 of *LDLR*. Total plasma cholesterol before treatment is shown below each symbol in mmol/l; to convert to mg/dl, multiply by 38.6. The patient's mother was married three times (symbols marked 1–3). Note that cholesterol levels in the patient's father and in his daughter by his second wife are much lower than those in the family of the patient's mother, but other unrelated patients with this same mutation (Asp461Asn) have severely increased plasma cholesterol (AK Soutar, unpublished data). (**B**) Mean plasma cholesterol levels in unaffected, heterozygous and 'homozygous' members of the same family. An unpaired *t*-test demonstrated a significant difference between plasma cholesterol levels of unaffected and heterozygous individuals. Abbreviations: Hmz, homozygous; Htz, heterozygous; Nor, unaffected individuals.

THE METABOLIC AND CELLULAR DEFECT

The underlying defect in FH was long thought to be caused by oversynthesis of cholesterol, but measurement of whole-body LDL metabolism revealed that the fractional catabolic rate of LDL was lower in heterozygous FH individuals than in normal subjects.[6] This difference was due to a defect in the mechanism for clearing LDL rather than a defect in LDL itself, and was not caused by saturation of normal mechanisms by the high serum levels of LDL present in FH individuals.[7] Thus, the stage was set for the eventual identification of the genetic defect in FH by Brown and Goldstein, with their discovery of the LDL receptor.[8,9] These investigators found that cholesterol synthesis was indeed raised in cultured skin fibroblasts from FH patients, but that this was the result of impairment of the downregulation that normally occurs when cells are incubated with serum. They showed that LDL was the specific serum component responsible for this rise but, crucially, they found that while cholesterol synthesis in FH cells was unaffected by incubation with LDL, it was reduced by incubation with pure cholesterol that, unlike LDL, could enter cells by simple diffusion. They deduced that the defect in FH cells must be the absence of a high affinity receptor for uptake of serum LDL.[8,9]

Brown and Goldstein went on to characterize the so-called 'LDL-receptor pathway' (Figure 2), revealing details of receptor-mediated endocytosis that not only had far-reaching implications for other pathways, but would subsequently enable identification of other genetic defects that caused malfunction of the LDL receptor.[10] They purified the LDL-receptor protein[11] and raised specific anti-LDL-receptor antibodies[12] that allowed them to confirm that multiple mutations cause FH.[13] They classified the cellular defects of LDL-receptor function into five groups: ligand-binding defective; transport defective; internalization defective; recycling defective; and 'null', which resulted in no detectable protein.[14] Finally, they cloned the LDL-receptor gene (*LDLR*)[15,16] and confirmed the identity of the first mutation that resulted in defective LDL-receptor function in FH,[17] subsequently showing that mutations in different parts of the gene affected receptor function differently.[13,14] Sequencing the gene allowed prediction of the presence of different domains in the protein, each encoded by separate exons or groups of exons, and often similar to parts of other apparently unrelated proteins, suggesting that the receptor might have evolved through shuffling of exons from other genes (Figure 3A).[16] Although it was simple to demonstrate that LDL-receptor activity in cultured cells maintained intracellular cholesterol homeostasis by sterol-mediated regulation of *LDLR* transcription, unraveling the precise mechanism revealed further novel and intriguing aspects of LDL-receptor molecular biology.[18]

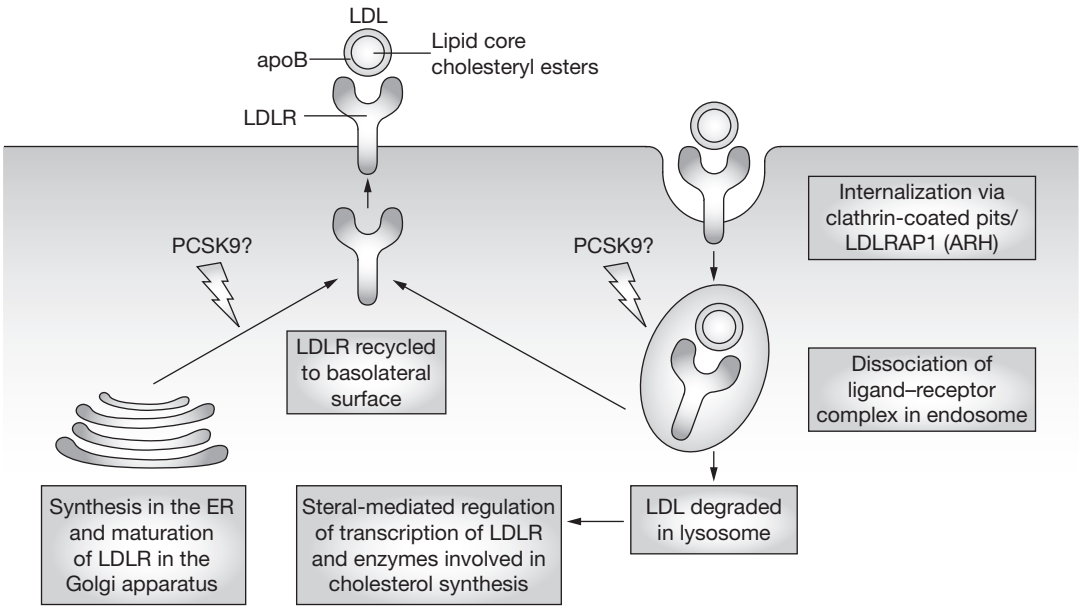

Figure 2 The LDL-receptor pathway for uptake and degradation of LDL. The LDL receptor is a cell-surface glycoprotein that is synthesized as an immature protein and processed in the Golgi apparatus, producing the mature form that is transported to the cell surface. There, the receptor specifically binds apolipoprotein B in LDL particles present in the extracellular fluid. The receptor–ligand complex is then internalized by endocytosis via clathrin-coated pits through interactions involving the LDL receptor adaptor protein, LDLRAP1 (also known as ARH). The complex is transported via early endosomes to the late endosomal compartment, where the acidic environment causes dissociation of the receptor–ligand complex. The receptor is recycled to the cell surface while the LDL particle is degraded in the lysosomal compartment. Accumulation of free cholesterol released by hydrolysis of cholesteryl esters in the core of LDL inactivates sterol regulatory element binding protein (SREBP), a transcription factor that drives expression of genes for enzymes involved in cholesterol synthesis and the LDL receptor. Thus the LDL-receptor pathway maintains intracellular cholesterol homeostasis. The proprotein convertase, PCSK9, normally reduces the LDL–protein content of cells by a post-translational mechanism that is not yet fully understood. Mutations in *LDLR, APOB, LDLRAP1* or *PCSK9* are known to result in familial hypercholesterolemia. Abbreviations: apoB/*APOB*, apolipoprotein B; ER, endoplasmic reticulum; LDLR, LDL receptor; LDLRAP1 (ARH), LDL receptor adaptor protein 1; PCSK9, proprotein convertase subtilisin/kexin type 9.

GENETIC CAUSES OF THE FH PHENOTYPE

Mutations in *LDLR*

Although analysis of LDL-receptor protein in cells had shown that the mutations causing FH in different patients must be heterogeneous,[13,14] the number of different mutations that have been identified is unprecedented. *LDLR* mutation databases currently list more than 800 different mutations (Figure 3C).[19–21]

The first few defects in *LDLR* to be characterized were large deletions identified by Southern blotting.[15] For this technique, patients' DNA was digested with different restriction enzymes and hybridized with the newly-isolated LDL-receptor complementary DNA probe labelled with ^{32}P, while identification of point mutations at that time required making a genomic library to enable cloning of the defective gene from each patient. Once amplification by PCR and direct automated sequencing of PCR products became possible, the relative ease of this as a means to identify single base changes in the gene compared with the more laborious Southern blotting has greatly increased the proportion of known point mutations and minor deletions/insertions relative to large rearrangements identified since then (Figure 3C). The advent of the new and simple method for detection of major gene rearrangements by quantitative PCR (multiplex ligation-dependent probe amplification)[22] will no doubt redress the balance.

Many different types of *LDLR* mutation have been identified in patients with FH worldwide, including large rearrangements, premature stop codons, single amino acid substitutions,

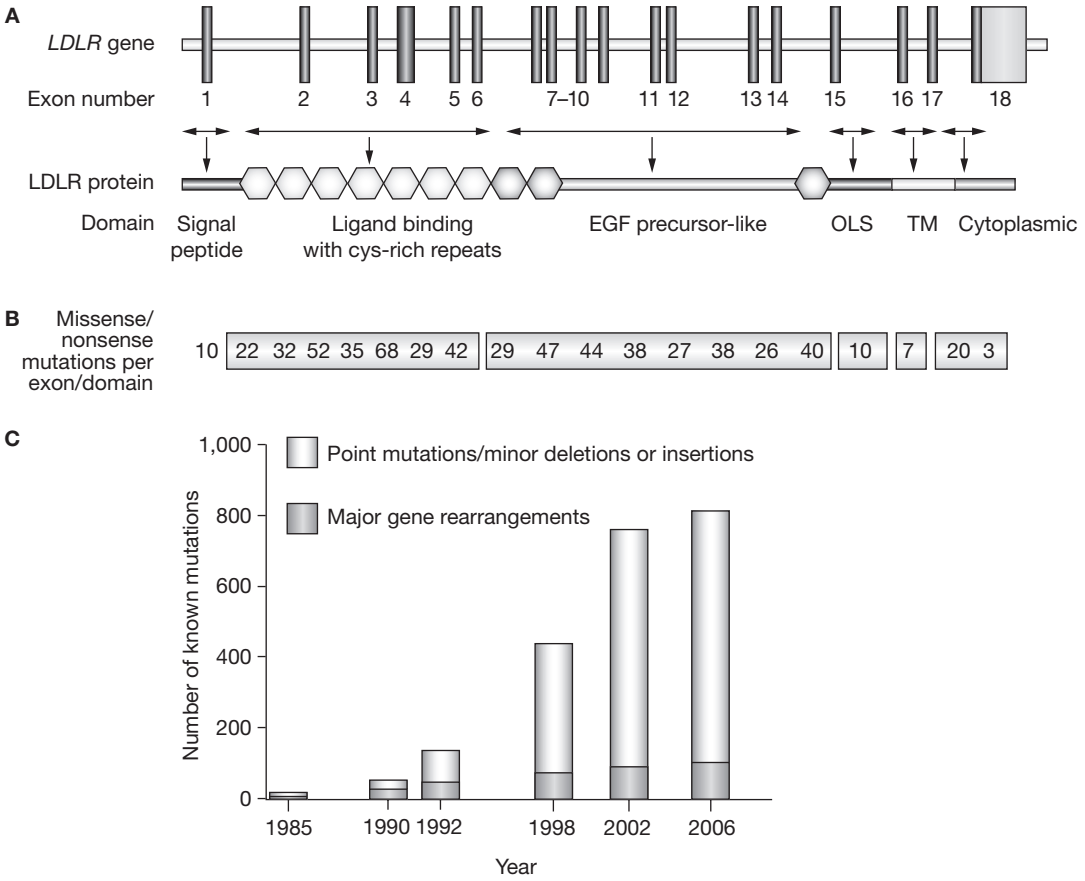

Figure 3 The *LDLR* gene. (**A**) The *LDLR* gene; exons are shown as vertical dark bars, numbered underneath. Single exons or groups of exons (indicated by horizontal arrows) encode the different domains of the LDL receptor protein. The cytoplasmic domain contains the peptide motif NPVY that is required for internalization of the receptor. (**B**) The number of point mutations in each exon or domain (boxed) that have been found in patients with familial hypercholesterolemia worldwide. (**C**) The number of known mutations in the *LDLR* gene. The *LDLR* gene was cloned in 1985, when the first mutations in patients with familial hypercholesterolemia were identified. The number of known mutations increased rapidly once gene amplification by polymerase chain reaction and direct automated DNA sequencing became available. Abbreviations: EGF, epidermal growth factor; LDLR, LDL receptor; OLS, *O*-linked sugars; TM, transmembrane.

mutations in the promoter region that affect gene transcription and mutations that affect splicing of the pre-messenger RNA (pre-mRNA).[19–21] Much information has been obtained about the structure and function of the LDL receptor from the effect of particular amino acid substitutions on the properties of the altered receptor in cultured cells. For example, the first point mutation to be identified was in the region encoding the cytoplasmic domain of the protein.[23] In cells from this patient, the receptor protein appeared to be normal but could not be internalized from the cell membrane, and this discovery led to the elucidation of the role of the NPVY internalization signal in clathrin-mediated endocytosis of the LDL receptor.

Interestingly, mutations in the LDL receptor that affect its function are spread fairly evenly throughout the *LDLR* gene (Figure 3B), and almost every single amino acid substitution that has been found has a deleterious effect, even though as many 'normal' alleles as dysfunctional alleles have been sequenced during analysis of heterozygous FH patients. There are, however, some notable exceptions,[24,25] and one of the problems with quick and easy sequencing is that sequence variants occasionally appear in the databases as deleterious mutations when their effect on LDL-receptor structure and function is open to question. Ideally, the putative mutant LDL receptor should be expressed in heterologous cells *in vitro* and its activity compared

with normal activity determined. Generally, the absence of a variant from a large number of normolipidemic individuals, its cosegregation with raised cholesterol in the patient's family, and conservation of the affected amino acid residue in LDL receptors from many species, together with the nature of the amino acid substitution itself, provide sufficient evidence that it is pathogenic.[25] Premature stop codons or substitutions of cysteine residues in cysteine-rich repeats always result in a completely nonfunctional protein, as do almost all large gene rearrangements. Variants that might affect splicing are more difficult to assess.[26] Base substitutions in the immediate splice junction adjacent to an exon usually affect splicing,[27] but not always,[28] and mutations can occur in the splice branch point that are up to 20–30 base pairs upstream of the exon.[29] In some cases a base change in an intron relatively distant from the exon can unmask a cryptic splice site,[30] but even though algorithms exist that 'test' a potential splice site, demonstration of the presence of abnormally spliced mRNA by reverse-transcription PCR of LDL-receptor mRNA from the patient's cells is essential to confirm that splicing is affected. Sometimes the presence of a 'silent' variant in the coding region can be useful to determine whether mRNA from both alleles is present in cells.

Familial ligand-defective apolipoprotein B

In the mid-1980s, Grundy and colleagues showed that turnover of LDL was reduced in some hypercholesterolemic patients who did not have all the hallmarks of heterozygous FH.[31] With Innerarity and colleagues, they showed that in some cases this finding resulted from an inherited defect in the ability of the patient's LDL to bind to the LDL receptor.[32] This binding occurs via the sole protein component of LDL, apolipoprotein B (apoB), but it was not known whether the defect was in apoB or whether differences in the lipid composition of LDL affected binding affinity. Finding the molecular defect was not easy because of the large size and polymorphic nature of apoB and the heterogeneity of the lipids in LDL particles. Eventually a point mutation in the apoB gene (*APOB*) was identified that was predicted to cause a single amino acid substitution of Arg3500 with glutamine (Arg3500Gln) in its proposed LDL-receptor-binding domain.[33] Most individuals with familial ligand-defective apoB are heterozygous for this allele, and a monoclonal antibody that distinguishes between normal and Arg3500Gln apoB has been used to show that LDL containing the mutant apoB accumulates in the circulation.[34] Furthermore, the mutant LDL has reduced ability to support the growth of U937 (human leukemic monocyte lymphoma) cells, which are dependent on LDL-receptor-derived cholesterol for normal growth in serum-free medium.[35]

Compared with individual mutations in *LDLR*, each of which is rare, this one mutant *APOB* allele described is common in Europe, where 2–5% of hypercholesterolemic patients are heterozygous for the defective allele. The penetrance of the mutant *APOB* allele, however, is not 100%, so patients with familial ligand-defective apoB have less-severe phenotypes than FH patients with *LDLR* mutations.[36,37] In one large systematic study in Denmark, the prevalence of Arg3500Gln apoB in the general population was 1/1,000 and the mutant allele was associated with significantly raised plasma cholesterol.[38] In Europeans, the mutation is believed to have been inherited on an allele of *APOB* with a rare haplotype from a single common ancestor who lived about 6,750 years ago,[39] although the same mutation seems to have recurred at least once.[40] Despite extensive searches, and in marked contrast to the LDL receptor, only one other mutation of the *APOB* gene has been found that affects its receptor-binding function: a substitution in the same codon as the first mutation, resulting in substitution of Arg3500 with tryptophan (Arg3500Trp).[41] This mutation is rare in Europe, but is relatively common in the Chinese population.[42] A third variant, Arg3531Cys (substitution of Arg3500 with cysteine), was found through screening of hypercholesterolemic individuals,[43] but later studies have shown that the frequency of this variant is the same in the general population as in hypercholesterolemic groups.[38]

Autosomal recessive hypercholesterolemia

In his first description of the genetics of FH, Khachadurian had noted that in a small number of families there seemed to be a recessive, rather than dominant, pattern of inheritance of severe hypercholesterolemia,[2] and in subsequent years other similar reports appeared. This disorder was referred to as autosomal recessive hypercholesterolemia (ARH) to

distinguish it from FH caused by *LDLR* mutations (Figure 4; reviewed by Soutar *et al.*[44]). Interestingly, no defect in LDL-receptor function in cultured skin fibroblasts from patients with ARH could be detected, despite all other characteristic signs of homozygous FH in the index patient. Thus, it was assumed at first that LDL-receptor function was not defective. When our research group identified its first 'clinically homozygous' FH patient with a recessive mode of inheritance and no detectable *LDLR* defect, however, we chose to study immortalized lymphocytes from the patient rather than skin fibroblasts, and found that although the LDL-receptor protein was produced normally, it failed to be internalized and thus LDL could not be taken up.[45] Subsequently, recessive null mutations in a novel gene called LDL receptor adaptor protein 1 (*LDLRAP1*; also known as *ARH*) were observed to cosegregate with hypercholesterolemia in members of these rare 'clinically homozygous' families.[46] We confirmed that these mutations were the cause of the FH phenotype by demonstrating that LDL-receptor function was restored in our patients' lymphocytes by expression of the normal LDL-receptor protein.[47] The LDLRAP1 (also known as ARH) protein contains a conserved phosphotyrosine-binding domain, and seems to function as an accessory adaptor protein that interacts with the LDL receptor via its cytoplasmic domain, enabling the receptor to engage with the clathrin-coated pit machinery for endocytosis (Figure 4C). Why LDLRAP1 is not required for LDL-receptor uptake in skin fibroblasts in culture is still unclear.

So far all known patients with ARH have mutations in *LDLRAP1* and, as expected for a recessive disorder, the majority of these patients have been the offspring of consanguineous parents, although one or two compound heterozygous cases are known.[44] All but one of the known mutations are null, in that no mutant protein can be detected in patients' cells, the exception resulting in a mutant protein lacking part of the critical phosphotyrosine-binding domain (Figure 4D). Presumably because of the founder effect, ARH is particularly common in Sardinia where there are three mutant alleles, one of which has resulted from recombination between the other two alleles.[3]

The phenotype in ARH is similar to that of patients with homozygous FH due to mutations in *LDLR*, but it is somewhat milder in terms

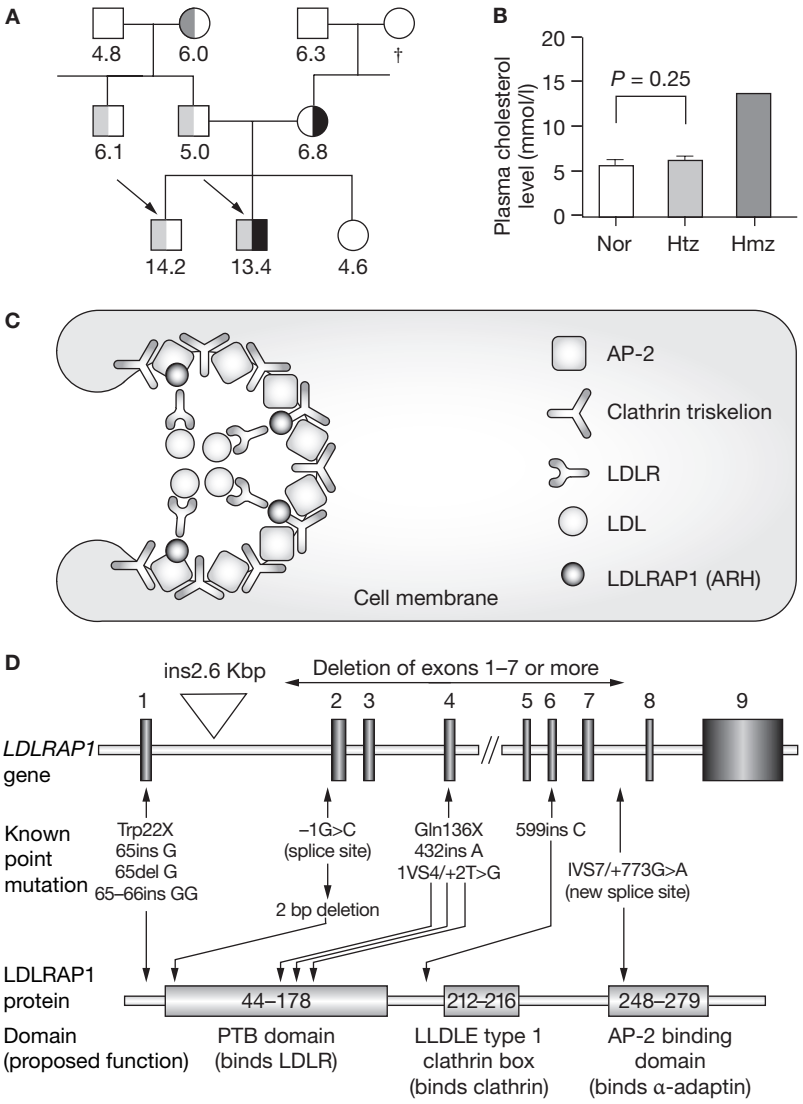

Figure 4 Autosomal recessive hypercholesterolemia. (**A**) The families of some rare patients with all the characteristics of homozygous familial hypercholesterolemia show a recessive mode of inheritance, as is the case with the two brothers (indicated with arrows) in this pedigree, one of the rare cases of autosomal recessive hypercholesterolemia in which two different mutant alleles have been inherited. The mutation inherited from the mother is a single base-pair insertion in exon 6, while that from their father is a large deletion encompassing exons 2–7. Total plasma cholesterol before treatment is shown below each symbol in mmol/l; to convert to mg/dl, multiply by 38.6. (**B**) The mean plasma cholesterol levels of unaffected, heterozygous and 'homozygous' members of the family. There was no significant difference between unaffected and heterozygous individuals in this family (by unpaired *t*-test). (**C**) Data indicate that the LDL receptor adaptor protein (LDLRAP1, or ARH) functions to engage the LDL receptor with the clathrin-coated pit machinery for endocytosis. (**D**) Diagram of the *LDLRAP1* gene. Exons are shown as vertical bars numbered above, with the position of known point mutations shown below and two large gene rearrangements. All mutations are 'null' and would result in a truncated protein, as indicated in the diagram of the protein. The different domains of the LDLRAP1 protein and their proposed function on the basis of studies *in vitro* are shown. Abbreviations: †, deceased; AP-2, adaptor protein 2; bp, base pair; Hmz, homozygous; Htz, heterozygous; LDLR, LDL receptor; LDLRAP1 (ARH), LDL receptor adaptor protein 1; Nor, unaffected individuals; PTB, phosphotyrosine binding.

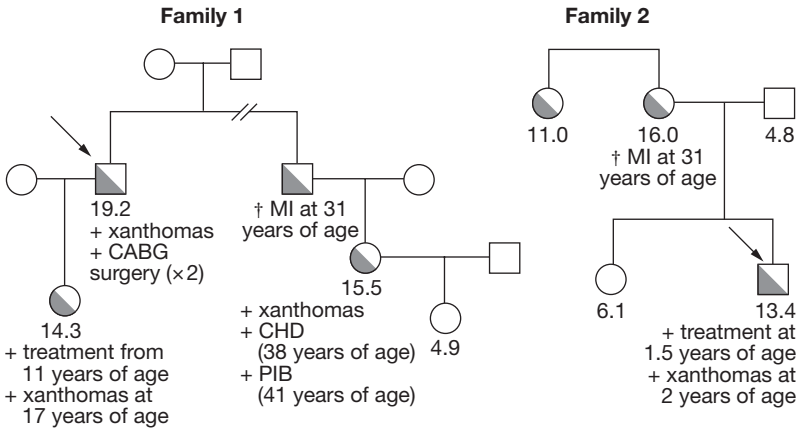

Figure 5 Severe phenotype in patients heterozygous for the Asp374Tyr mutation in the PCSK9 protein. Pedigrees of two families in which the Asp374Tyr mutation in PCSK9 cosegregates with severe hypercholesterolemia and premature coronary heart disease are shown. Long before their genetic defect was known, all these patients had been identified as 'hard to treat'. Total plasma cholesterol before treatment with lipid-lowering drugs is shown below each symbol in mmol/l; to convert to mg/dl, multiply by 38.6. Abbreviations: †, deceased; CHD, coronary heart disease; MI, myocardial infarction; PIB, partial ileal bypass.

of serum total cholesterol and LDL cholesterol levels, and shows better response to treatment with lipid-lowering drugs with or without LDL apheresis. The phenotype also tends to be more variable within affected individuals from the same family.[4] In addition, serum HDL cholesterol levels in patients with ARH are appreciably higher than those in patients with homozygous FH.[48] The development of premature CHD is, therefore, delayed in individuals with ARH, and no CHD has been documented in patients younger than 20 years, unlike FH homozygotes of whom >40% of individuals younger than 20 years develop CHD (the overall odds ratio for CHD in FH homozygotes versus ARH is 9.1; 95% CI 4.4–19.1).[48] Some, but not all, obligate heterozygote ARH carriers showed higher serum total cholesterol and LDL cholesterol levels than noncarrier family members.[4,48]

Mutations in *PCSK9*

First found in French families,[49] mutations in *PCSK9*, a gene that encodes a putative protease named proprotein convertase subtilisin/kexin type 9 (PCSK9), have since been shown to cosegregate with severe hypercholesterolemia in a number of families in several countries,[50–53] but remain a rare cause of FH.[54] One particular mutation, Asp374Tyr, seems to have arisen in the early Nordic population, as it is now relatively common in Norway and in the UK, where it is associated with a particularly severe clinical phenotype (Figure 5).[5] The mechanism by which mutations in *PCSK9* cause hypercholesterolemia is not yet fully understood, but *PCSK9* is a sterol-regulated gene,[55,56] indicating involvement in cholesterol metabolism. Adenoviral-mediated overexpression of normal (or mutant) *PCSK9* has been shown to reduce LDL-receptor protein levels in the liver of mice.[57,58] This finding led to the interesting discovery that two loss-of-function truncating mutations in *PCSK9*, which occur in the US black population at a frequency of about 1/50, are associated with both markedly lower than normal plasma LDL levels and strong protection against early CHD (Figure 6).[59] Under normal circumstances, therefore, the PCSK9 protein reduces hepatic LDL-receptor activity; loss-of-function mutations impede this reduction. The underlying mechanism of the gain-of-function mutations, however, remains unclear. Some evidence indicates that apoB secretion might be increased in addition to a reduction in LDL-receptor activity,[51,60] and LDL with an abnormal composition has been observed in patients heterozygous for mutant *PCSK9*.[5] Much effort is now focused on understanding this mechanism, and on searching for variants of *PCSK9* that influence plasma LDL cholesterol levels, but *PCSK9* is highly polymorphic and most common variants seem to be silent.[61] Clearly, inhibition or repression of *PCSK9* expression is a new and important target for the pharmaceutical industry.

Other candidate genes

Despite all the improvements in mutation detection technology, when groups of clinically diagnosed FH patients are screened for *LDLR* mutations there are always some in whom no mutation can be found. The presence of mutations in other candidate genes has been postulated, but these are very rare. For example, a recessive mutation in *CYP7A1*, which encodes the enzyme that catalyses the first step in the hepatic catabolism of cholesterol, has been found as a recessive trait in a single family,[62] but no other families with a defect in this gene have come to light. Variants in the genes for regulatory proteins such as SREBP-2 (SRBP2) or SCAP have been found in patients with FH,[63,64] but the evidence that these variants cause the phenotype is not strong.

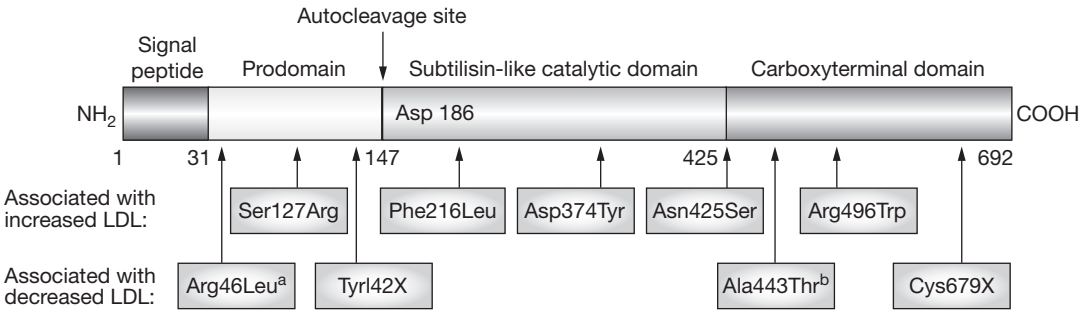

Figure 6 PCSK9 variants associated with plasma LDL cholesterol concentration. Diagram of the PCSK9 protein, showing the position of the domains with homology to the prodomain and the catalytic domain (with the Asp–His–Ser catalytic triad) of the subtilisin family of proprotein convertases. The third domain has no known homology. Immediately below the protein are indicated the variants that cosegregate with hypercholesterolemia in familial hypercholesterolemic families; the evidence of pathogenicity is stronger for the variants in bold type. Below these are shown the variants for which there is convincing evidence that they are significantly associated with lower than normal plasma LDL cholesterol levels.[59] [a]Only associated with low cholesterol in whites. [b]Only associated with low cholesterol in American blacks homozygous for the variant.

CLINICAL VALUE OF IDENTIFYING THE GENETIC CAUSES OF FH

Variability of the phenotype

The clinical phenotype of FH is highly variable, and it was first assumed that this variability depended on the level of residual LDL-receptor activity associated with each particular mutant *LDLR* allele. Although homozygous FH patients with some residual LDL-receptor function are considerably less severely affected clinically than those with no detectable LDL-receptor activity,[1] when sufficient numbers of patients are studied this association does not seem to hold true for adult heterozygous patients,[65] despite many attempts to prove otherwise. Children with heterozygous FH with a null genotype, however, have higher LDL cholesterol levels and greater carotid intima-media thickness than children with receptor-defective mutations, which indicates that they might benefit from more-aggressive treatment started in childhood.[66] Furthermore, the clinical phenotype in carriers of the *APOB* Arg3500Gln mutation is also variable, despite the presence of the same mutant allele.[36] Missense *PCSK9* mutations lead to very different serum LDL cholesterol levels in the affected individuals, ranging from relatively mild to severe elevations;[49–52] the most severely affected are those with the Asp374Tyr mutation.[5]

Only one or two *LDLR* mutations can genuinely be considered 'mild' (i.e. all carriers of the mutation have milder hypercholesterolemia than most patients with FH).[65,67] For all other patients with FH caused by *LDLR* defects, environmental or other inherited factors seem to be more important than the type of mutation in determining the phenotype severity.[68] These are often the same factors that predispose to CHD in the general population (i.e. smoking, low HDL cholesterol level, male sex and increasing age). It has been suggested that there is not necessarily a strong correlation between the extent of cholesterol increase and the incidence of CHD in individuals with FH,[68] but the importance of the raised LDL cholesterol level in FH is demonstrated by our study of Chinese heterozygous FH patients.[69] We found that individuals who had migrated to Canada were severely hypercholesterolemic and had the same CHD risk as other patients with FH, whereas their counterparts still living in China had no CHD, and mild hypercholesterolemia. In fact, heterozygous FH 'patients' in China only came to light as obligate carriers of *LDLR* mutations because they were parents of homozygous offspring. Many of the mutant *LDLR* alleles were the same, and haplotype analysis showed that they had probably been inherited from common ancestors. Thus, the difference in cardiovascular risk must be due largely to environmental differences.[69]

Numerous efforts have been made to show that variation at other gene loci can affect the clinical phenotype of FH in patients with *LDLR* mutations, but most of these have been inconclusive because of the small numbers of patients studied. Although it is likely that such variants do exist,[70] it could be that many of these will

be rare alleles that can have a marked effect in an individual pedigree,[71–73] but might not be readily detectable in groups of FH patients by single-nucleotide-polymorphism association studies.[74] Of course, there could also be alleles of genes that interact with environmental factors to influence the phenotype, but examples have not yet been found in FH.

Response to lipid-lowering therapy

Although the hypercholesterolemia associated with FH can be controlled with cholesterol-lowering drug therapy (statins and ezetimibe), patient response can vary quite widely. It was once believed that the response might depend on the type of *LDLR* mutation, but this does not seem to be the case, as two patients with the same mutation can respond differently.[75] Patients with hypercholesterolemia caused by mutations in genes other than *LDLR*, however, respond differently from patients with *LDLR* mutations. In patients with ARH, for example, response to treatment with lipid-lowering drugs, singly and in combination, is more pronounced than in patients with homozygous FH caused by *LDLR* mutations.[4,76] Interestingly, same-sex siblings with ARH can differ significantly in their response to treatment.[4] The opposite is seen in family members with the Asp374Tyr gain-of-function *PCSK9* mutation, who all require high doses of combination lipid-lowering therapy to achieve substantial cholesterol reductions.[5]

Perhaps the main benefit of being able to identify the mutations that cause FH, or indeed any genetic disorder, is that this allows genetic diagnosis in families of patients. Population screening for FH is unlikely to be cost-effective, even though compared with the calculated frequency of the disorder only a small proportion of FH patients are currently being diagnosed and appropriately treated. The occurrence of so many different *LDLR* mutations and their widespread distribution throughout the gene imposes severe practical limitations on simple genetic screening. Indeed, exon-by-exon sequencing of *LDLR* and other genes in each index patient is considered the screening method of choice,[77] although some researchers carry out a preliminary screen to identify exons with a potential variant.[78] A microchip able to detect 40 different *LDLR* mutations has been developed in a region of Spain,[79] but it would detect only half of the mutations detectable by sequencing in patients from another area of the country.[80] A full resequencing chip for *LDLR* would be ideal, but is prohibitively expensive at present.

Cascade screening

Cascade screening in extended families of known index patients with FH is the most efficient means of identifying hitherto undiagnosed affected individuals, and several countries have instigated such programs at the national level.[81] One unresolved question is whether the index patients for cascade screening should be those with a known molecular defect[81,82] or those diagnosed using clinical criteria, as in the recently initiated cascade screening in the UK.[83] The pros and cons of the two approaches have recently been discussed.[84] Although the prevailing opinion at present seems to be that genetic analysis should be limited to founder populations in which only a few *LDLR* mutations account for most of the cases, genetic screening has increased the percentage of affected relatives receiving treatment from 39% to 93% in The Netherlands[81] and from 53% to 89% in Norway.[82] At present there is no single internationally accepted clinical diagnosis of FH. Development of age-related and sex-related LDL cholesterol cutoff levels that are sensitive and specific for each region for diagnosis of affected relatives in cascade screening might lead to greater accuracy in identifying FH patients in countries with non-founder mutations or where there are technical or financial constraints on genetic analysis.[84] Importantly, screening based on clinical criteria will not exclude families with no detectable mutation but with high cholesterol levels who would benefit from early diagnosis and treatment.

Screening for genetic disease in children is a sensitive issue and is under special ethical regulations in different countries. In The Netherlands only children older than 16 years of age can take part in the national genetic screening program (J Defesche, personal communication), whereas in Norway screening is performed in younger children after parental consent is obtained, as it is assumed that the test result will have therapeutic implications.[82] Indeed, more than a third of Norwegian children with FH have total serum cholesterol levels greater than 8 mmol/l (309 mg/dl), placing them at high risk of premature CHD. Reassuringly, several studies have confirmed that the majority of parents with FH were willing for their

children to be screened and that it did not have a negative psychological impact.[81,85] The main clinical concerns following FH diagnosis in children or adolescents include if or when to start statin treatment and what the target cholesterol levels should be. After careful evaluation of the individual's risks and benefits, only children with the highest risk of future CHD should be considered for statin treatment.

CONCLUSIONS

Elucidation of different genetic defects in patients with FH has lead to a better understanding of the LDL-receptor pathway and its role in lipoprotein metabolism, and has also produced some surprises in the form of novel genes, the products of which carry out novel cellular functions. The discovery of *PCSK9* has reinforced the view that maintaining low plasma LDL cholesterol levels from an early age is vital to reduce CHD risk, and has revealed a new target for lipid-lowering therapy.

KEY POINTS

- Familial hypercholesterolemia (FH) is usually inherited as an autosomal dominant disorder caused by defective clearance of LDL from the circulation that occurs with a frequency of about 1/500
- Pioneering studies by Brown and Goldstein revealed that FH was usually caused by defective function of a cell-surface receptor for LDL caused by mutations in *LDLR*
- Subsequent work has revealed that there are numerous different mutations in the LDL-receptor protein, but these do not explain the variability in the severity of clinical symptoms in heterozygous patients, and the fact that environmental factors are also important
- Not all patients with clinical FH have mutations in *LDLR*; mutations in *APOB*, *PCSK9* and *LDLRAP1* can affect LDL-receptor function *in vivo*
- Diagnosis of FH is important to reduce premature coronary heart disease, but genetic screening for FH is hampered by the large number of different mutations
- Cascade screening of affected relatives of index patients has already increased rates of diagnosis and treatment of FH in some countries; population screening, however, is probably not cost-effective

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Competing interests
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