GENETIC STUDIES OF FAMILIAL HYPERCHOLESTEROLEMIA: A REVIEW

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ABSRTACT

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.

Key words: Amilial hypercholesterolemia, LDLR gene, apolipoprotein B-100, autosomal recessive hypercholesterolemia, sitosterolemia, cholesterol 7-Hydroxylase deficiency, PCSK9.

BACKGROUND

Familial Hypercholesterolemia (FH) is characterized clinically by an increased level of circulating LDL cholesterol that leads to lipid accumulation in tendons and arteries, premature atherosclerosis and increased risk of coronary heart disease (CHD) (Austin *et.al,* , 2004).FH is an autosomal dominant disorder affecting approximately 1 in 500 people (10 million world-wide) and the elevated serum cholesterol concentration leads to more than 50% risk of fatal or non-fatal coronary heart disease by the age of 50 years in men and at least 30% in women aged 60 years. FH usually results from inherited defects in low density lipoprotein receptor gene (LDLR) or by mutations in the gene encoding apolipoprotein B (APOB). The most recently identified gene causing FH is one encoding a member of the proprotein convertase family, PCSK9.

More than 1000 mutations have been identified in the LDLR gene. The presence of mutations in other candidate genes has been postulated, but these are very rare. For example, a mutation in CYP7A1, which encodes the enzyme that catalysis the first steps in the hepatic catabolism of cholesterol, has been found as a recessive trait.

DISEASE
Familial Hypercholesterolemia

Familial Hypercholesterolemia (FH) is an autosomal dominant disorder affecting the regulation of cholesterol homeostasis. Clinical and biochemical features of FH include xanthomata, premature coronary heart disease (CHD) and elevated plasma cholesterol (Goldstrein *et.al,* 1995). Most FH-related mutations identified to date are located in the coding region of the low-density lipoprotein receptor (LDLR) gene or at the intron: exon junction, while mutations in the promoter region appear to be rare (Top *et al.,* 1992; Hobbs *et. al.,* 1992; Day *et al.,* 1997 and Varret *et al.,* 1998). This review will describe the genetic basis of FH and the defects in different genes found to result in the same clinical phenotype.

GENETIC CAUSES OF THE FH PHENOTYPE
LDLR gene

The molecular genetics of FH has proved to be complex. The human LDLR gene located on chromosome 19 comprises 18 exons that span a distance of about 45 kb. The essential regulatory elements of the LDLR gene lie within ~200 bp upstream of the transcription initiation site, and three imperfect direct repeats are largely responsible for promotor activity (Südhof, 1987). Repeats 1 and 3 bind Sp1, a *trans*-acting transcription factor, which promotes transcription of the LDLR gene in the presence and absence of sterols (Südhof, 1987; Dawson, 1998). The 10 bp core sequence of repeat 2, designated as the sterol regulatory element (SRE-1), is essential for high levels of

transcription of the LDLR gene (Smith *et. al.,* 1991; Briggs, 1993 and Koivisto *et. al.,* 1994). In the case of sterol depletion, SRE-1 interacts with the essential transcription binding proteins (SREBP) to induce transcription of the LDLR gene (Yokoyama *et al.,* 1993; Oliner, 1996 and Streicher *et al.,* 1996) whilst responsible for sterol-mediated repression of the gene when cellular cholesterol levels are high (Smith *et. al.,* 1991; Briggs, 1993). Mutations in the core of repeat 2 have been shown to reduce transcription significantly only in the absence of sterols (Smith *et. al.,* 1991; Briggs, 1993). Two additional *cis*-acting regulatory elements have recently been identified and are designated foot printing 1 (FP1) and foot printing 2 (FP2) elements. FP1 and FP2, spanning nucleotide intervals -145 to -126 and -187 to -175, respectively, are deemed essential for maximal induction of transcription. It was suggested that FP1-induced transcription might be through interaction with SRE-1 (Dhawan *et.al,,* 1997). A variety of substances, such as cytokines, growth factors and hormones, have also been reported to influence regulation of transcription of the LDLR gene. Most of the mutations in FH patients have been identified in the LDLR gene, since this gene has been studied in detail and is responsible for the majority of cases of FH. However, this review shall focus on all the genes which are known to be involved in FH.

Apolipoprotein B-100 gene

Familial defective apoB-100 (FDB), results from a missense mutation (Arg3500 Glu) in the LDLr-binding domain of apo B-100 (Soria *et.al,* 1989). Like FH, elevated plasma LDL-C levels with normal TG, tendon xanthomas, and premature atherosclerosis characterize FDB.

The FH clinical phenotype can also result from the mutations in the apoplipoprotein B-100 (Soria *et. al.* 1989 and Innerarity *et al.,* 1990), located on chromosome 2p23-24 (Knott *et al.,* 1986). Two mutations in APOB (R3500Q and R3500W) have been identified in studies of individual with a clinical FH phenotype (Soria *et. al.,* 1989).

LDL particles are catabolized primarily via the LDLR pathway, apolipoprotein moieties of the LDL particles, and the hepatic LDLRs. Apo B molecules are synthesized in hepatocytes and secreted in VLDL, where they function as ligands for the LDLR after conversion of VLDL to LDL. Binding of apo B to the LDLR results in cellular internalization of the LDL particles and their lysosomal degradation (Brown and Goldstein, 1989). Hence, on one hand, defects of the LDL receptor as observed in FH or, on the other hand, defects of the ligand as observed in FDB results in increased LDLC concentrations (Innerarity *et al.,* 1990 and Brown and Goldstein, 1989). Defective binding of LDL particles to their receptors due to structural defects of apo B was recently identified in patients with moderately increased concentration of LDLC (Innerarity *et al.,* 1990; Weisgraber *et al.*, 1988 and Vega and Grundy, 1986). This metabolic disorder was designated as FDB. A markedly reduced (20% to 32% of normal) binding of LDL particles isolated from subjects with FDB to LDLRs of cultured fibroblasts and a subsequent decrease of the clearance of LDL have been demonstrated in this disorder (Innerarity et al., 1990 and Marz *et al.,* 1993).

FDB is caused by a single base substitution (G to A) at nucleotide 10,708 (Knott *et al.,* 1986) in exon 26 of apoB gene, creating an arginine-to-glutamine substitution at the codon for amino acid 3500 (Soria *et. al,* 1989).

Autosomal Recessive Hypercholesterolemia gene

Autosomal recessive hypercholesterolemia (ARH) is a recessive disorder; most of the patients are homozygous for the same allele. ARH is caused by mutation in the ARH gene also called LDLRAP1, which encodes a novel adaptor protein (Garcia et al., 2001). To date, ten ARH mutations have been described (Cohen, 2003 and Eden et al., 2002). So far, all the described mutations in ARH are predicted to introduce premature stop codons, either as a result of a point mutation or a frameshift.

The gene encodes a protein ARH that contains a phosphotyrosine-binding (PTB) domain similar to that found in many adaptor proteins that are known to bind NPXY motifs in the cytoplasmic domain of signaling receptors (Forman-Kay and Pawson, 1999).

Before the characterization of ARH, it was known that LDL receptors promote endocytosis of LDL by clustering within clathrin-coated regions on the plasma membrane.
Characterized by the polygonal lattice composed principally of assembled clathrin trimers and the adaptor protein-2 (AP-2) complex, these coated regions invaginate to form an intracellular transport intermediate, the clathrin-coated vesicle.

Sorting of receptor proteins into clathrin-coated vesicle is selective and the minimal sequence necessary for efficient uptake of the LDL receptor is FDNPVY, located within the cytoplasmic domain (Cohen, 2003). The importance of the FXNPXY internalization motif first emerged when a point mutation in the tyrosine residue (Y807C) was identified in an FH patient whose cells in culture exhibited impaired internalization of the LDL receptor (Davis *et.al.*, 1986).

The transferrin receptor is efficiently sorted into coated vesicles by binding directly to AP-2, but its internalization sequence differs from that of the LDL receptor (Banifacino and Traub, 2003), and there is good

evidence that these two receptors use separate sorting mechanisms to enter clathrin coats (Norman et.al., 1999; Wilund et al., 2002 and Warren *et.al*, 1998). Thus, the identification of ARH immediately suggested that it was a likely candidate for mediating specific interactions of the LDL receptor with the endocytosis machinery.

The most striking feature of the 308-amino acid ARH protein is an amino- terminal PTB domain. The presence of PTB module immediately suggest that ARH interfaces directly with the LDL receptor because the usual binding partner of this domain is an FXNPXY consensus sequence (Yan *et. al.,* 2002 and Stolt *et.al.,* 2003), although, ARH is clearly required for normal LDL receptor-mediated endocytosis in hepatocytes, in a much as these cells are the major site of LDL receptor-mediated clearance of LDL in vivo. Failure of the liver to take up and degrade plasma LDL provides the only plausible explanation for the severe accumulation of LDL in the circulation observed in ARH patients.

PCSK9 gene

PCSK9 is the proprotein convertase subtilisin/kexin type 9a, also designated as NARC-1 (neural apoptosis-regulated convertase 1). NARC-1 is a novel putative proprotein convertase belonging to the subtilase subfamily. A related protein is the subtilisin kexin isoenzyme-1 (SKI-1)/site-1-protease (S1P), which has a key role in cholesterol homeostasis through processing the sterol regulatory element-binding proteins. The cDNA spans 3,617 bp and encodes a protein of 692 amino acids (Abifadel *et al.,* 2003).

PCSK9 belongs to the proteinase K subfamily of subtilases. PCSK9 is synthesized first as a soluble zymogen that undergoes autocatalytic intramolecular processing in the ER to produce a prosegment that remains associated with the secreted enzyme.

Two missense mutations in PCSK9 were found to cosegregate with an autosomal dominant form of hypercholesterolemia by linkage studies in two large French FH families. The first mutation results in the substitution of an arginine for serine (S127R). The second missense mutation (F216L), also in a conserved amino acid, is in the catalytic domain of the enzyme (Park *et.al.,* 2008).

PCSK9 acts through a post-transcriptional mechanism to negatively regulate LDLR protein levels. Overexpression of PCSK9 associated with hypercholesterolemia in humans by reducing LDLR protein, resulting in an increase in plasma levels of LDL cholesterol. The mutations causing hypercholesterolemia causes a gain of function in PCSK9 that ultimately results in hypercholesterolemia (Park *et. al.,* 2008).

Other candidate genes

In sitosterolemia, plasma levels of sitosterol are elevated more than 50-fold and comprise approximately 15% of circulating and tissue sterols. Sitosterol is derived from plants and shellfish. Patients with sitosterolemia can develop aortic stenosis and premature coronary atherosclerotic disease. A distinctive clinical feature of sitosterolemia is the occurrence of low-level hemolysis, presumably due to the incorporation of plants sterol into red blood membranes. Sitosterol, though differing from cholesterol by only minor modifications of the side chain, has a very different metabolic fate. Normally, both sitosterol and cholesterol are taken up into enterocytes in the proximal small intestine, and between 20% and 80% dietary cholesterol is incorporated into chylomicrons. In contrast, less than 5% of the dietary sitosterol is absorbed. The small amount of sitosterol that is transported to the liver is preferentially secreted into the bile. Patients with sitosterolemia have increased fractional absorption of dietary sterols and a defect in the ability to secrete sterols into the bile, resulting in the accumulation of both animal and plant sterols in the blood and body tissues (Salen *et. al.,* 1970; 1992).

Sitosterolemia results from a defect in sterol efflux from cells. It is caused by mutations in either of the two adjacent genes that encode ABC half transporters, ABCG5 and ABCG8 (Berge *et al.,* 2000 and Lee et al., 2001). More than 25 different mutations cause sitosterolemia (Berge *et al.,* 2000; Lee *et al*., 2001 and Lu *et al.,* 2001)

Sitosterolemia patients invariably have two mutant alleles of ABCG8 or two mutant alleles of ABCG5; none of the patients identified has one mutation in ABCG5 and one in ABCG8.

ABCG5 and ABCG8 are expressed almost exclusively in hepatocytes and enterocytes.

Cholesterol 7-Hydroxylase Deficiency gene (CYP7A1)

CYP7A1 is the first enzyme in the classical pathway for bile acid biosynthesis (Pullinger *et al.,* 2002). Patients with CYP7A1 deficiency were homozygous for a frame shift mutation that results in premature termination of translation and a nonfunctional enzyme.

CYP7A1 deficiency presumably causes hypercholesterolemia by reducing hepatic LDLR activity.

CONCLUSIONS

Familial Hypercholesterolemia is caused by mutations of the low-density lipoprotein receptor gene. FH homozygote characterized by markedly elevated LDL cholesterol levels, tendon xanthomata and increased risk of coronary artery disease. The genetic basis of this disease is complex. Further understanding of the genetic basis of FH will result from the identification of other potential genes for the FH phenotype, including the apolipoprotein B-100 gene (APOB), the autosomal recessive hypercholesterolemia gene (ARH), PCSK9 and other candidate genes.

LIST OF ABBREVIATIONS:

FH, familial hypercholesterolemia; LDLR, low density lipoprotein receptor gene; APOB, apolipoprotein B-100; ARH, autosomal recessive hypercholesterolemia, CYP7A1 cholesterol 7-hydroxylase deficiency, PCSK9, proprotein subtilisin/kexin type 9a.

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