# Monogenic Hypercholesterolemias

# 10

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## Definition and Classification

Monogenic hypercholesterolemias (MHs) are a heterogeneous group of single-gene defects with Mendelian transmission in the family characterized by elevated plasma low-density lipoprotein (LDL) cholesterol levels and very high risk for premature atherosclerotic disease, especially coronary heart disease (CHD) [1] (Table 10.1).

Approximately, one in 200–500 people is affected by MH in most populations explored so far, so this group of diseases is among the most frequent genetic metabolic defects [2]. The study of MH has provided decisive evidence of the linkage between high LDL cholesterol concentration and atherosclerosis development in humans. Furthermore, the metabolic and genetic characterization of MH in the past decades has supplied crucial information about the cholesterol homeostasis, metabolism, and regulatory pathways. The scientific information generated around the MH has contributed decisively to the development of many drugs in common use today, such as hydroxy-methyl-glutaryl coenzyme A reductase (HMG-CoAR) inhibitors or statins, which have contributed to change the evolution of arteriosclerotic disease. The information generated around the MH remains very active today, and the discovery of new genes responsible for high LDL cholesterol is promoting the development of very promising new drugs, such as inhibitors of proprotein convertase subtilisin/kexin type 9 (PCSK9), and others.

MH traditionally included two common diseases of autosomal dominant inheritance: familial hypercholesterolemia (FH), due to mutations in the LDL receptor gene (*LDLR,* Online Mendelian Inheritance in Man (OMIM) 143890) causing isolated high LDL cholesterol (type IIa hyperlipoproteinemia) and familial combined hyperlipidemia (FCHL) of unknown etiology (OMIM 144250) usually associated with mixed hyperlipidemia secondary to high concentrations of very LDLs (VLDL) and LDL particles (type IIb hyperlipoproteinemia); as well as several rare recessive diseases such as sitosterolemia (OMIM 210250) and autosomal recessive hypercholesterolemia (ARH;OMIM 603813) [3]. However, FH is heterogeneous from the genetic standpoint, and mutations in the *LDLR* are found only in 60–80 % of patients with a clinical diagnosis of FH [4]. Functional mutations in other genes produce indistinguishable clinical phenotypes of FH, including a missense mutation p.(Arg3527Gln) located in the LDLR-binding domain of

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**Table 10.1** Monogenic hypercholesterolemias (MHs) causing high LDL cholesterol

| Inheritance | Disease name | Defective gene | Prevalence[a] | Plasma LDL cholesterol (mg/dL) |
|---|---|---|---|---|
| *Dominant* | | | | |
| | Familial hypercholesterolemia (FH) | *LDLR* | 1 in 500 | 200–500 (heterozygous) 500–800 (homozygous) |
| | Familial defective apo B-100 | *APOB* | 1 in 2000 | 200–400 (heterozygous) 500–800 (homozygous) |
| | FH3 | *PCSK9* | <1 in 10,000 | 200–500 (heterozygous) |
| | FH4 | *APOE* | ? | 200–500 (heterozygous) |
| | Hyperlipoproteinemia(a) | *LPA* | 1 in 2500 | 200–300 |
| | Autosomal dominant familial combined hyperlipidemia | *LDLR, C5L2, APOE, PCSK9,?* | 1/500 | 200–350 |
| *Recessive* | | | | |
| | Autosomal recessive hypercholesterolemia | *LDLRAP1* | <1 in $10^6$ | 400–600 |
| | Sitosterolemia | *ABCG5/ABCG8* | <1 in $10^6$ | Variable |
| | Lysosomal acid lipase deficiency | *LIPA* | <1 in 50,000 | 200–300 |
| | Cholesterol 7-alpha-hydroxylase deficiency | *CYP7A1* | Very rare | 150–210 |

[a] Some prevalences are highly heterogeneous among populations. *LDL* low-density lipoprotein

apolipoprotein (apo) B-100 that produces familial defective apo B-100 (FDB, OMIM 144010) [5]; gain-of-function mutations in PCSK9, a protein that binds to the LDL receptor inducing its degradation along with the LDL particle, and are termed FH3 (OMIM 603776) [6, 7]; and, a deletion in a codon of APOE (p.Leu167del) has been recently associated with autosomal dominant hypercholesterolemia in two different studies [8, 9]. Very high levels of lipoprotein(a) ((Lp(a)), named as hyperLp(a), is a single-gene condition also causing MH [10]. Except for the presence of apo(a) in the LDL particle surface, Lp(a) is essentially indistinguishable from LDL, so Lp(a) carries in some cases substantial amounts of cholesterol. Lp(a) varies from 0.1 to 300 mg/dL among individuals due to the *LPA* gene locus which codes for the apo(a) [11]. *LPA* kringle IV-2 sequence is present in a variable number of identical repeated copies (from 3 to >60) and the number of kringle IV-2 repeats is inversely correlated with the Lp(a) concentration [12]. Lp(a) is discussed in extension in another chapter of this book.

The genetic heterogeneity of isolated high LDL cholesterol in MH, the clinical similarities among them, which make their clinical diagnosis in most cases indistinguishable, their common high cardiovascular risk, and their uniform response to the different lipid lowering treatments, mean that all of them are referred to collectively as FH, regardless of the presence of mutations in the *LDLR* [13, 14]. Hence, FH should be defined as a group of monogenic genetic defects resulting in severely elevated serum LDL cholesterol concentrations with autosomal codominant transmission pattern of inheritance.

In contrast, most cases of FCHL, the most common genetic form of hyperlipidemia identified in survivors of myocardial infarction [15, 16], do not correspond to a monogenic disease, rather they are complex genetic diseases resulting from the interaction of multiple genetic and environmental factors mainly overweight, obesity, saturated fat- and sugar-enriched diets, and physical inactivity [17]. Many families with FCHL combine adipose tissue dysfunction [18, 19], insulin resistance [20], hepatic overproduction of VLDL particles [21], and peripheral slow clearance of triglycerides-rich lipoproteins [22]. Different association and linkage studies have shown more than 40 different genes associated with FCHL that have been recently reviewed by Brouwers et al. [23] although with great differ-

ences among studies. Therefore, FCHL is currently considered to be a complex phenotype consequence of multiple genetic defects, each one mostly with minor effects, which differ among families, and among populations. In over 50% of the families with the clinical diagnosis of FCHL, all effected members are overweight or obese, and with high frequency they develop diabetes mellitus with time [24]. This predisposition within certain families, to develop mixed hyperlipidemia only in the presence of increased body fat deposits, we have proposed to be named as "adiposity-related familial hyperlipidemia" [25]. However, the actual definition of FCHL, familial transmission of high apo B levels with high plasma total cholesterol and/or triglycerides, also includes some forms in which the lipid phenotype is largely determined by a single gene [23]. These less common forms of autosomal dominant FCHL demonstrate that, in some cases, the FCHL phenotype is largely determined by a single genetic defect, and behaves as an MH [8, 26] (Table 10.1).

## Familial Hypercholesterolemia

As defined above, FHs are a group of monogenic genetic defects resulting in severely elevated serum LDL cholesterol concentrations with autosomal codominant transmission pattern of inheritance. Hence, patients with two defective alleles (FH homozygotes or compound heterozygotes) have much higher LDL cholesterol than those with one mutant allele (FH heterozygotes). Lifelong elevated plasma concentrations of LDL cholesterol are responsible for the major clinical manifestation of FH: premature CHD and extravascular cholesterol deposits as tendon xanthomas or corneal arcus [27]. The frequency of FH heterozygotes (1 in 500 individuals) is much higher than FH homozygotes (<1 in 1 million). However, some populations such as French Canadians [28], Afrikaners in South Africa [29], Lebanese, and Finns [30] have a much higher prevalence due to a founder effect.

## Etiology and Pathogenesis

FH caused by mutations in *LDLR* was the first disease of lipid metabolism to be genetically defined, the best known at present time, and the most frequent MH in most countries around the world. Different mutations in the LDLR affect the LDL receptor protein functionality [27]. LDL receptor in cell membranes binds LDL and the complexes enter the cell by endocytosis [31]. The LDL receptor is synthesized as a 120-kDa precursor protein, which is converted to a mature form of apparent molecular mass of 160 kDa. The increase in molecular mass is correlated with extensive *N*- and *O*-glycosylation in the Golgi apparatus during transfer to the cell surface [32]. In addition to the glycosylation, in the endoplasmic reticulum (ER), the 21-amino-acid signal peptide of the LDL receptor is cleaved to give rise to a mature receptor. The transmembrane LDL receptor (glycoprotein of 839 amino acids) is present at the surface of most cell types and mediates the transport of lipoproteins containing apo B or apo E into cells, through receptor-mediated endocytosis. The mature LDL receptor reaches the cell surface and is directed towards clathrin-coated pits where it binds to apo B- and apo E-enriched lipoproteins via its extracellular domain [33]. The lipoprotein–LDL receptor complex is endocytosed and migrates to endosomes. At the acidic pH of the lysosomes, LDL is released, allowing LDL receptor to return to the membrane and entering into a new cycle [34] (Fig. 10.1). Although the LDL receptor was initially thought to play the single role of helping to achieve cholesterol homeostasis, its expression in neurons suggests it may also play other functional roles [35, 36].

Cholesterol homeostasis is among the most regulated processes in biology. Cellular cholesterol balance is achieved by both synthesis and uptake through LDL receptor. When cellular cholesterol levels rise, *LDLR* transcription is reduced and de novo synthesis is inhibited. When cellular cholesterol storage is depleted, *LDLR* transcription is activated and de novo synthesis activated. Two major transcriptional *LDLR* regulation pathways have evolved in mammals to
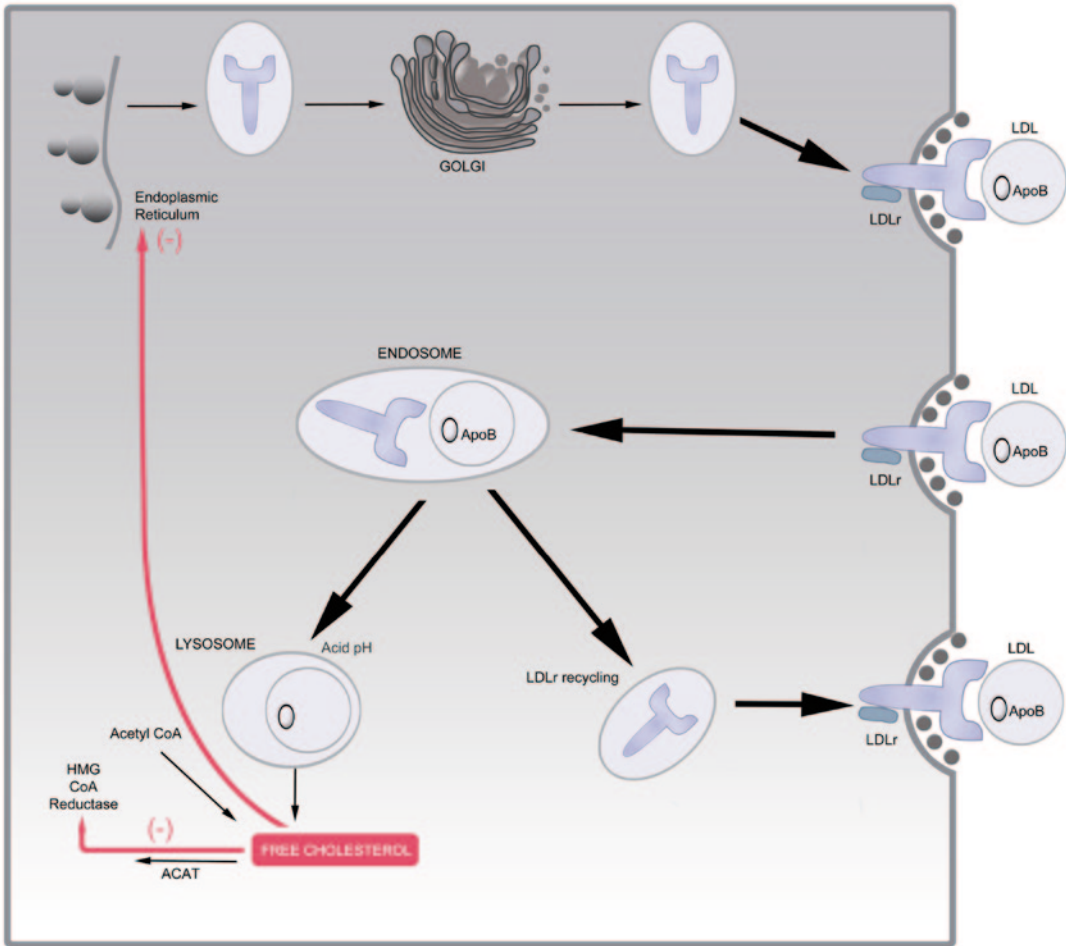
**Fig. 10.1** Schematic representation of the itinerary of the low-density lipoprotein (LDL) receptor in human cells. The LDL receptor is synthesized in the endoplasmic reticulum (ER) as a precursor of apparent molecular weight of 120 kd and transported at the Golgi complex where the N-linked carbohydrates are processed. Once transferred to the surface of the cell, the receptor recognizes the apolipoprotein B-100 component of the LDL. Binding leads to cellular uptake and lysosomal degradation of the LDL by receptor-mediated endocytosis. This uptake process satisfies the cholesterol needs of the cells, and hence keeps cholesterol synthesis suppressed

coordinate responses to both elevated and reduced cellular cholesterol content: the sterol regulatory element-binding proteins (SREBPs) and the liver X receptors (LXRs). SREBP-2 promotes the expression of the *LDLR,* thereby increasing LDL uptake and cholesterol delivery to cells [37]. The SREBP-2 precursor protein resides in the ER and is transported to the Golgi apparatus under low intracellular cholesterol content, where it undergoes proteolytic processing. The mature SREBP protein translocates to the nucleus and switches on the transcription of *LDLR,* as well as other genes involved in cholesterol biosynthesis, including HMGCoAR and HMGCoA synthase (HMGCoAS) [38]. An additional modulator of LDL receptor-dependent cholesterol uptake independent of the SREBP pathway is the LXR [39]. LXR induces expression of E3 ubiquitin ligase inducible degrader of the LDL receptor (Idol), which in turn catalyzes the ubiquitination of the LDL receptor and targets it for degradation [40].

Characterization of Idol-deficient cells has also provided insights into to the functional relationship between PCSK9 and Idol pathways. PCSK9 and Idol share the same protein substrates, but PCSK9 is still able to induce LDL receptor degradation in Idol$^{-/-}$ cells, suggesting that Idol and PCSK9 may be complementary but independent pathways [41]. PCSK9 is secreted into plasma and binds to the first domain (EGF-A) of epidermal growth factor (EGF) homology repeats of LDL receptor [42–44]. Although the C-terminal domain of PCSK9 is not required for LDL receptor binding, it is required for LDL receptor degradation [33]. The complete mechanism by which PCSK9 binding to the LDL receptor targets the receptor for degradation is not understood. Although PCSK9 is a protease, it does not cleave LDL receptor, nor is the proteolysis of LDL receptor required to downregulate *LDLR*. The LDL receptor–PCSK9 complex is internalized via clathrin-mediated endocytosis and then routed to lysosomes via a mechanism that does not require ubiquitination and is distinct from the autophagy and proteosomal degradation pathways [45].

Posttranscriptional regulation of *LDLR* expression is also a major determinant of lipoprotein metabolism. LDLR adaptor protein 1 (LDLRAP1) is a protein required for the efficient activity of LDL receptor. It has been demonstrated that LDLRAP1 is essential for the efficient internalization of the LDL–LDL receptor complex and cells from patients with ARH fail to internalize the LDL receptor because they carry two defective alleles of LDLRAP1, a gene that encodes a specific clathrin adaptor protein [46]. LDLRAP1 is an endocytic sorting adaptor that actively participates in the internalization of the LDL–LDL receptor complex, possibly enhancing the efficiency of its packaging into the endocytic vesicles [47]. LDLRAP1 is required not only for internalization of the LDL-LDL receptor complex but also for efficient binding of LDL to the receptor. LDLRAP1 stabilizes the associations of the receptor with LDL and with the invagination portion of the budding pit, thereby increasing the efficiency of LDL internalization [48].

## The *LDLR* Gene

The *LDLR* is mapped to chromosome 19p13.1-13.3 and spans 45 kb and contains 18 exons and 17 introns encoding the six functional domains of the mature protein: signal peptide, ligand-binding domain, EGF-like, O-linked sugar, transmembrane, and cytoplasmic domain [49, 50] (Fig. 10.2). The human LDLR complementary DNA (cDNA) and gene were cloned and characterized in 1984 and 1985, respectively [51, 52]. The gene sequencing of the *LDLR* suggested that the LDL receptor is a mosaic protein built up of exons shared with different proteins, and it therefore belongs to several supergene families [52].

Exon 1 encodes a hydrophobic sequence of 21 amino acids that correspond to the signal peptide, which is cleaved from the protein into the ER during the translocation process. Around 4.5 % of the total *LDLR* mutations described including frameshift, missense, and nonsense sequence variants have been located in this exon (http://www.ucl.ac.uk/fh).

Exons 2–6 encode the ligand-binding domain, a cysteine-rich sequence of seven tandem structurally homologous repeats of 40 amino acids each, which is responsible for binding lipoproteins. The structure of the ligand-binding domain has been partially elucidated. Each repeat contains a cluster of negatively charged amino acids, Asp-X-Ser-Asp-Glu and six cysteine residues that form three disulfide bonds [53–55]. Binding of lipoproteins to the LDL receptor appears to be mediated by an interaction between acidic residues in the LDL receptor binding domain and basic residues of apo E and apo B-100 [53, 56]. Repeats R3–R7 are necessary for LDL binding (apo B-100-mediated), whereas remnant lipoproteins binding (apo E-mediated) is impaired only when R5 is deleted. Repeats R4 and R5 are sufficient to bind to apo E-phospholipids vesicles [54]. We have proposed a new mechanism for the release of LDL particles in the endosome; it is based on the instability of repeat 5 at endosome low pH and low $Ca^{2+}$ [57]. Under these conditions, repeat 5 is unable to bind $Ca^{2+}$ and appears in an unfolded conformation not expected to bind
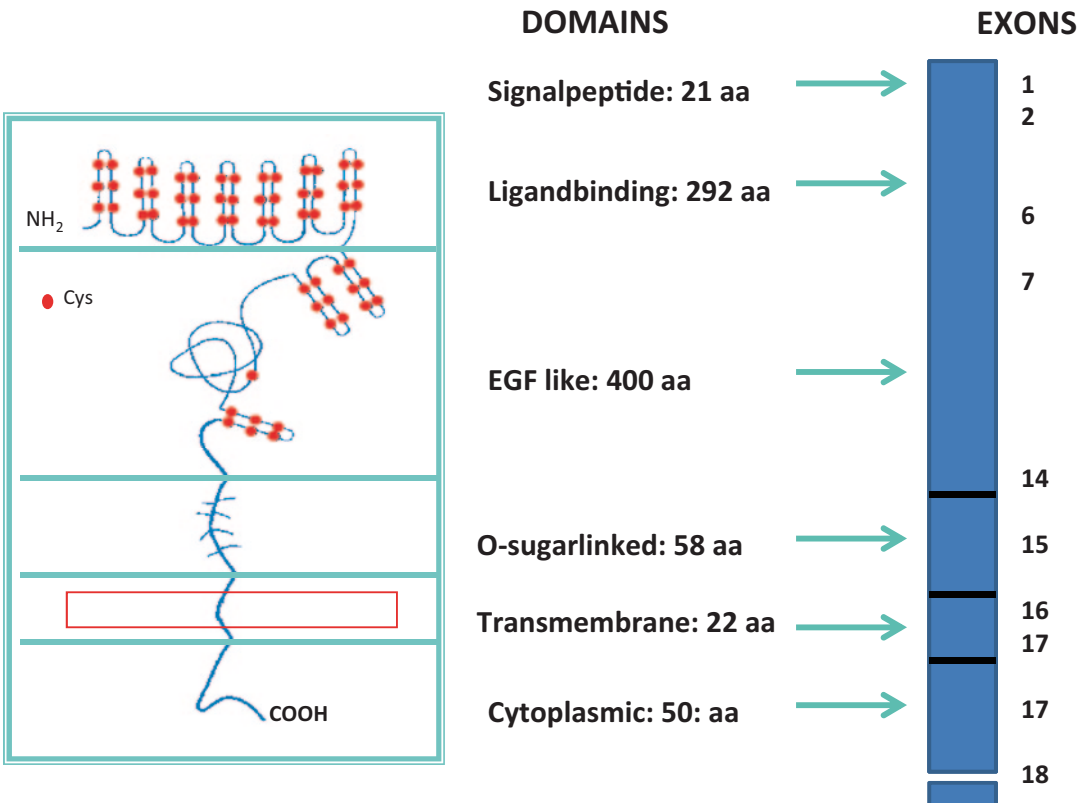
**Fig. 10.2** Schematic representation of the five domains in the structure of the human low-density lipoprotein (LDL)-receptor protein and their corresponding exons in the LDLR gene. *Red dots* represent the six cysteine residues that form three disulfide bonds in each tandem structurally homologous repeats or class A repeats in the ligand-binding domain of the LDL receptor protein

LDL particles. In the ligand-binding domain, 40 % of the total allelic variants associated with FH have been found to date (http://www.ucl.ac.uk/fh).

Exons 7–14 encode a region that shares 33 % sequence identity to the human EGF gene. This domain consists of a 411-amino-acid sequence, encoded by exons 7–14. Like the ligand-binding domain, this region also contains three repeats of 40–50 amino acids with cysteine-rich sequences. The first two repeats, designated A and B and encoded by exons 7 and 8, are contiguous and separated from the third repeat, C encoded by exon 14, by a 280-amino-acid sequence that contains five copies of the conserved motif Tyr-Trp-Thr-Asp, encoded by exons 9–13. PCSK9 binds to EGF-A repeat, decreasing receptor recycling

and increasing degradation [43]. The EGF-like domain is required for the acid-dependent dissociation of the LDL particles from the LDL receptor and clathrin-coated pits during receptor recycling. When the EGF-like domain is deleted from the LDL receptor, the receptor can no longer bind LDL particles but it still binds lipoproteins that contain apo E [58]. The majority of FH mutations described (55 % of total) have been associated with the EGF homology region.

Exon 15 encodes an LDL receptor domain of 58 amino acids rich in Thr and Ser residues. The function of this domain is unknown, but it has been observed that this region serves as an attachment site for O-linked carbohydrate chains and it is thought that it plays a role in the stabilization of the receptor. This domain shows

minimal sequence conservation among six species analyzed, and Davis et al. reported that deletion of clustered O-linked carbohydrates does not impair function and turnover of human LDL receptor in Chinese hamster ovary (CHO) cells transfected with the human *LDLR* gene [59]. However, from the analysis of LDL receptors in CHO mutant cells with defective uridine diphosphate (UDP)-galactose and UDP-*N*-acetylgalactosamine 4-epimerase, Kingsley et al. proposed that O-linked carbohydrate chains may be crucial for receptor stability [60]. A total of 41 allelic variants within exon 15 are registered in *LDLR* databases.

Exon 16 and the 5′-end of exon 17 encode a domain of 22 hydrophobic amino acids that is essential for anchoring the LDL receptor to the cell membrane.

The cytoplasmic domain of the LDL receptor, that compromises 50-amino-acid residues, is encoded by the remainder 3′ region of the exon 17 and the 5′ end of the exon 18 [27]. This domain contains two sequence signals for targeting the LDL receptor to the surface and for localizing the receptor in coated pits [61]. This domain is the most conserved region of the LDL receptor, which is more than 86 % identical among six species [27]. Only a few allelic variants, 6 % of the total, have been identified within these domains.

The DNA motifs essential for the transcriptional regulation of the LDLR are located within 177 bp of the proximal promoter. The LDL receptor production is tightly regulated by a sophisticated feedback mechanism that controls the transcription of the *LDLR* in response to variations in the intracellular sterol concentration and the cellular demand for cholesterol [62]. The promoter region contains all the *cis*-acting elements for basal expression and sterol regulation and includes three imperfect direct repeats of 16 bp each, repeats 1–3. Repeats 1 and 3 contain binding sites for Sp1 transcription factor, and contribute to the basal expression of the gene, requiring the contribution of the repeat 2 for a strong expression. Repeat 2 contains a regulatory element, sterol regulatory element (SRE)-1, that enhances transcription when the intracellular sterol concentration is low through interaction with SREBP

[63]. Several naturally occurring mutations have been mapped to the transcriptional regulatory elements of the *LDLR*.

Nowadays, over 1500 naturally occurring *LDLR* mutations have been described in FH patients (http://www.ucl.ac.uk/fh). The *LDLR* mutations can produce defects in transcription, posttranscription processes, translation, and posttranslation processes. FH mutations have been classified into five classes depending on phenotypic behavior of mutant protein [64]. Class 1 mutations are known as "null alleles," which fail to produce immune-precipitable LDL receptor protein. Most of them are due to *LDLR* promoter deletion, rearrangements, frameshift, nonsense, or splicing mutations in a way that messenger RNA (mRNA) is not produced [64]. Class 2 mutations are transport-defective alleles which encode for proteins that cannot adopt an adequate tridimensional structure after being synthesized and keep them blocked, completely or partially (2A and 2B, respectively) in transport process between ER and Golgi apparatus. This defect is caused, usually, by missense mutations or small deletions in *LDLR* avoiding partially or completely the folding of the protein. These mutations are the most common at the *LDLR* locus [64]. Class 3 mutations are binding-defective alleles which encode for LDL receptor that are synthesized and transported to cell surface but fail to bind LDL particles. This is a heterogeneous group, because LDL binding activity goes from 2 to 30 % of normal. This defect is due to rearrangements in repeat cysteine residues in binding ligand domain or repeat deletions in EGF-like domain [65]. Class 4 mutations are known as internalization-defective alleles. These alleles produce proteins that are unable to cluster into clathrin-coated pits, therefore LDL receptor is not internalized [66]. Finally, class 5 mutations result in receptors that are able to bind and internalize LDL, but they fail to release LDL in the sorting endosomes and fail to recycle. Instead, they are rerouted to the lysosomes for degradation [67, 68].

Several studies have shown that different mutations are associated with differences in lipid levels, and it is likely that these will be associated with clinically different effects [29, 69]. In

addition, the phenotypic effect of the mutation is modulated by other genetic or environmental factors [29, 70]. Even the LDL lowering effect of statins in FH patients may depend on the nature of the *LDLR* mutation [71, 72]. Our group observed that FH patients with a molecular diagnosis show different advanced carotid and femoral atherosclerosis in relation to *LDLR* mutational class, thus FH patients with null allele mutations of *LDLR* show a more severe clinical phenotype and worse advanced carotid atherosclerosis than those with receptor-defective mutations, independently of age, gender, lipid, and nonlipid risk factors [73, 74].

## *APOB* Gene

*APOB* was the second locus identified to be responsible for MH. A group of individuals with a clinical phonotype similar to FH and also reduced LDL catabolism were found to have normal LDL receptor activity. The disease was secondary to a defective apo B that displays low affinity for the LDLR, and was named FDB. FDB is as common as FH in some European populations [75, 76] but much higher in Old Order Amish living in the USA [77]. The interaction between LDL and the LDL receptor is essential for the regulation of plasma cholesterol in humans. Apo B-100, the major protein component of LDL, is also a ligand for the LDL receptor; therefore, apo B-100 mediates the binding of LDL particle to the LDL receptor [78]. Studies using immunoelectron microscopy have shown that the N-terminal 89 % of apo B-100 enwraps the LDL particle like a belt and that the –COOH terminal 11 % constitutes a bow that crosses over the belt, bringing residues 4154–4189 and 4507–4513 close to amino acid 3527 [79].

Vega and Grundy observed that a group of patients with hypercholesterolemia have reduced clearance of LDL because of a defect in the structure or composition of LDL that reduces its affinity for receptors [80]. Innerarity et al. found that this type of hypercholesterolemia could be attributed to a defective receptor binding of a genetically altered apo B-100 to the LDL receptor [81].

The first mutation found in *APOB* as FDB cause was demonstrated by Soria et al. They observed a mutation in the codon for amino acid 3527 that results in the substitution of Gln for Arg (p.R3527Q) [5]. So far, ten true mutations at the *APOB* locus have been identified that alter the binding properties of apo B-100 indicating that FDB is more heterogeneous than previously assumed. Two mutations causing FDB were described in 1995: a change of Gln for a Trp in the amino acid 3527 (p.R3527W) and a substitution of Arg for Cys in 3558 codon (p.R3558C) [82, 83].

The binding affinities of p.R3527Q and p.R3558C to the LDL receptor are reduced to 30 and 70 %, respectively. Nevertheless, the deleterious effect of the p.R3558C variation was reconsidered not sufficient to cause hypercholesterolemia suggesting that it is more a susceptibility variation than a causative mutation [84].

The p.E3432Q mutation binds to LDL receptor at the same rate as normal LDL, but LDL particles containing this mutant protein are taken up and degraded at significantly reduced rates [85]. The *APOB* mutation p.R3507W has been associated with FDB because of its position near Trp 4396 that was shown to interact with Arg 3527 and facilitate the protein conformation required for normal receptor binding of LDL [86].

Another *APOB* mutation was found in codon 3543, located between known FDB mutations at codons 3527 and 3558, this mutation, p.N3543K, introduces a positively charged amino acid Lys, while other FDB mutations remove a positively charged residue Arg. The p.N3543K mutation influences conformation of LDL apo B and its interaction with the LDL receptor [82].

Other four *APOB* mutations, p.H3570Y, p.R3527L, p.R4385H, and p.V4394L were detected in hypercholesterolemic patients [87, 88]. However, the causative effect of these four mutations has not been yet demonstrated. Recent data reveal that compared with FH patients with *LDLR* mutations, FDB patients have lower LDL cholesterol levels by 20–25 %, respond better to statins, and have lower risk of CHD [88, 89]. This difference could be due to normal clearance of VLDL remnants through apo E-mediated uptake in FDB [90].
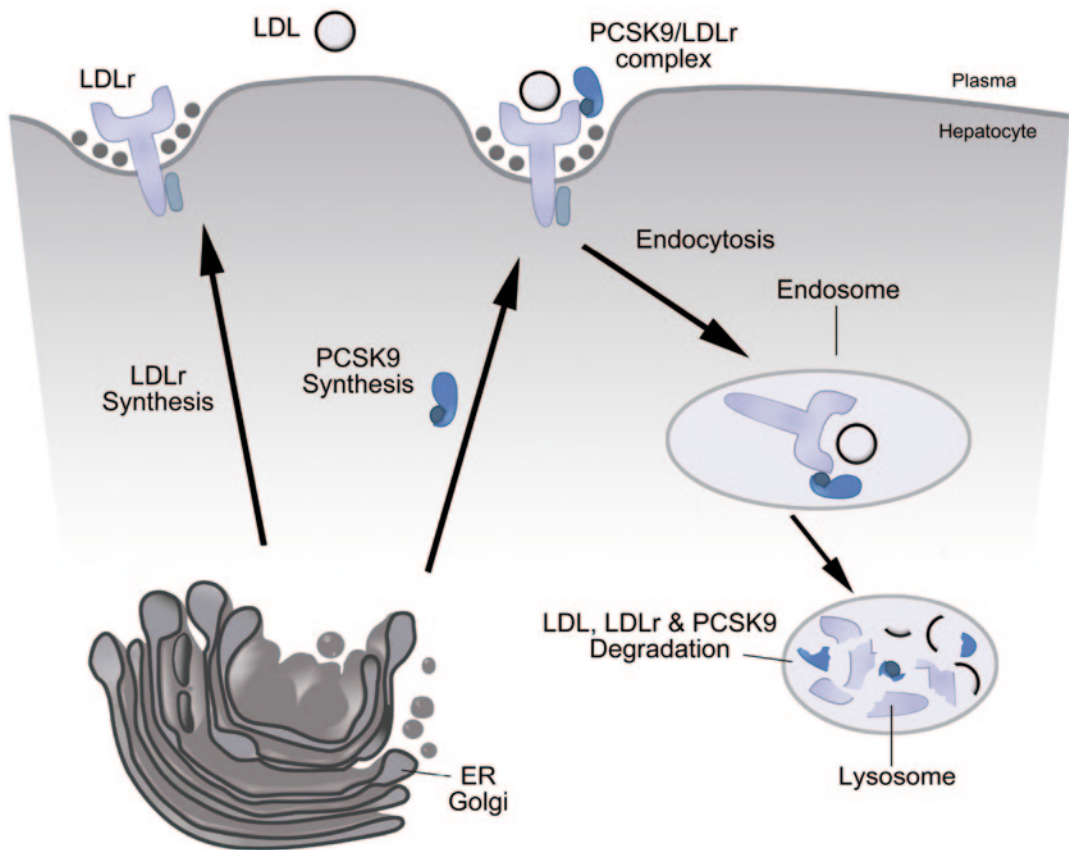
**Fig. 10.3** Proprotein convertase subtilisin/kexin type 9 (PCSK9) is involved in the metabolism of low-density lipoprotein (LDL) receptor. PCSK9 is synthesized in hepatocytes and released into blood. On the hepatocyte surface PCSK9 binds to the LDL receptor. The binding of LDL particles with the complex PCSK9/LDL receptor produces its internalization by endocytosis and lysosomal degradation of the LDL receptor. In the absence of PCSK9, LDL receptor degradation does not occur and the LDL receptor is recycled back to the cell surface

## Proprotein Convertase Subtilisin/Kexin Type 9 Gene

In 1999, Varret et al. identified by linkage analysis a third major autosomal dominant locus (HCHOLA3) at 1q34.1-p32 chromosome and showed that HCHOLA3 was in fact *PCSK9* [6, 91]. The *PSCK9* gene comprises 12 exons transcribed into a cDNA that spans 3617 bp. PCSK9 was first identified as a member of proprotein convertase family with hepatic, intestine, and kidney expression. PCSK9 is a 692-amino-acid glycoprotein that contains a 22-residue signal sequence followed by a pro-domain and a catalytic domain that shares structural homology with the proteinase K family of subtilisin-like serine proteases [7]. PCSK9 is a secreted protein that promotes degradation of the LDL receptor, and variants in *PCSK9* gene that cause hypercholesterolemia in humans are gain-of-function mutations [3, 92].

Initially, it was thought that PCSK9 has a role in LDL receptor degradation at the cell surface [92]. As we have described above, there is enough evidence to think that PCSK9 participates in LDL receptor lysosomal degradation via a mechanism that does not require ubiquitination and is distinct from the autophagy and proteosomal degradation pathways [45] (Fig. 10.3).

**Fig. 10.4** Effect of proprotein convertase subtilisin/kexin type 9 (PCSK9) mutations on plasma low-density lipoprotein (LDL) cholesterol concentration (Modified from reference [96])
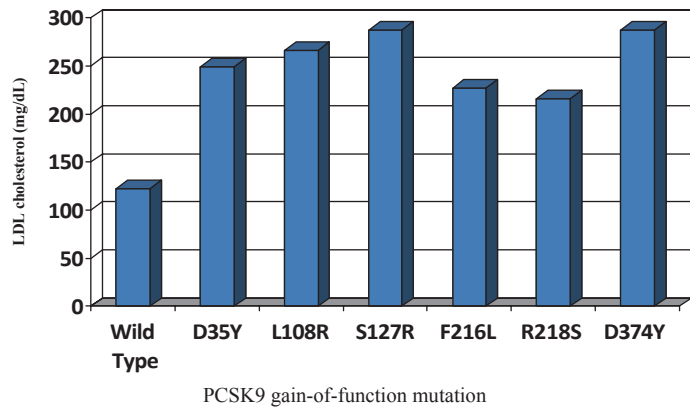
*PCSK9* mutations have been also classified into five classes, including "null alleles," mutations that affect autocatalytic scission, avoiding the protein transport through ER or from the ER to cell surface, alleles that affect PCSK9 stability and, finally, mutations that produce gain of function because of gene overexpression [93–96] (Fig. 10.4). By contrast, mutations in *PCSK9* that produce loss of function (Y142X, C679X, and R46 L) are associated with low LDL cholesterol [97, 98].

hyperlipidemia [8]. The mechanism of high LDL cholesterol associated with this mutation is unknown, but is predicted to interrupt an alpha-helix in the binding domain of apo E and reduce the catabolism of particles containing apo E, including LDL [9].

Several genome-wide linkage scan have suggested susceptibility FH *loci* on chromosomes 3q25–26, 8q24.22, 16q22.1, and 21q22. However, no gene nor disease-causing mutation was identified in these loci so far [104–106].

## Other FH *Loci*

The proportion of individuals with autosomal dominant hypercholesterolemia without a mutation in LDLR, APOB, or PCSK9 ranges from 12 to 60% (ADH-) [88, 99–102]. This variability is due mainly to the clinical–biological criteria used to select FH subjects as well as due to ethnicity [103]. Our group has demonstrated that hyperLp(a) is responsible for FH phenotype in approximately 6% of nonLDLR/nonAPOB subjects [10].

More recently, two groups independently have demonstrated that a rare mutation in *APOE,* c.500_502delTCC/p.Leu167del causes a lipid phenotype indistinguishable from classical FH in Spain and France [8, 9]. In the Spanish study, the mutation was found in probands with the clinical diagnosis of FCHL, but the family studies demonstrated that the most common phenotype in mutation carriers' family members was isolated high LDL cholesterol rather than combined

## Clinical Findings

**Atherosclerotic Vascular Disease in FH** Approximately 80% of FH heterozygotes and almost 100% of homozygotes will suffer and die of atherosclerosis vascular disease if they are not treated to lower their LDL cholesterol during long periods of time [107]. Symptomatic atherosclerosis disease presents as CHD before age 55 and 60, in over 50% of FH heterozygotes, men and women, respectively, while homozygotes with much higher LDL cholesterol typically suffer CHD very early in life and usually die before age 20 without treatment. In homozygotes, atherosclerosis begins in the aortic root, causing CHD and supravalvular aortic stenosis [27]. The mean age of onset of a cardiovascular event in men with heterozygous FH is in the early 40s and in women with FH in the early 50s. Approximately, 85% of males will suffer a coronary event before 65 years if they are not treated. Atherosclerotic vascular disease in FH is mostly CHD. FH repre-

**Table 10.2** Major CVD risk factors in heterozygous FH subjects

| Risk factor | Cut points |
| --- | --- |
| Age | Men >30 years of age |
|  | Women >40 years of age |
| LDL cholesterol | >250 mg/dL |
| Smoking | Current smoker |
| Family history of premature CHD | First-degree male relative <age 55 |
|  | First-degree female relative <age 65 |
| Blood pressure | >140 mm Hg systolic or >90 mm Hg diastolic |
| Low HDL cholesterol | <40 mg/dL |
| High lipoprotein(a) | >50 mg/dL |
| Physical findings | Tendon xanthoma |
| Diabetes mellitus | Presence |
| Genetic defect | LDL receptor-negative mutations |

*LDL* low-density lipoprotein, *CHD* coronary heart disease, *HDL* high-density lipoprotein

sents 1–2 % of all premature (<55 years of age in men and <65 years of age in women) myocardial infarctions in most countries [13, 14], and up to 9 % of total premature CHD in Eastern Finland [108] and Germany [109] are caused by FH. The mechanism of this excess of coronary lesions in FH with respect to other vascular beds such as lower limbs or carotid arteries is not known, but is probably related to the type of LDL particle which accumulates in plasma.

Risk factors associated with CHD in heterozygous FH are the traditional risk factors for the general population, but however, the effect of each risk factor is greater in FH [2, 13]. Besides, FH specific clinical or molecular features as tendon xanthomas or receptor-negative or null mutations in the *LDLR* gene have also been reported to increase risk of CHD in FH heterozygotes. Major risk factors for CVD in heterozygous FH are presented in Table 10.2 [2, 13]. The presence or absence of these factors modifies the LDL cholesterol treatment goals in FH [13].

**Extravascular Cholesterol Deposits** The presence of tendon xanthomas is common in people over age 40 in FH heterozygotes and almost constant in the first decade of life in FH homozygotes. The most characteristic location of tendon xanthomas is the Achilles tendon, but they are also common in elbows and fingers (Fig. 10.5). The presence of tendon xanthomas is associated with an increased risk of premature CVD, especially in women [110], and increased risk of

tendinitis. In recent years, due to the availability of effective treatments for hypercholesterolemia from youth, the prevalence of tendon xanthomas has dropped sharply. Achilles tendon sonography improves the detection of xanthomas, and facilitates the clinical diagnosis [111] (Fig. 10.6).

The corneal arcus in the first decades of life is another surface lipid deposition characteristic of FH (Fig. 10.7), which is sometimes used as a criterion in some diagnostic algorithms.

**Coronary Diease-Genotype Correlations in FH** Because of the large number of allelic variants (more than 1500) differently affecting LDL receptor clearance function, *LDLR* allele-specific differences may be predicted for the FH phenotype. *LDLR* mutations may be classified into different functional types: (1) receptor-negative or null alleles, which include disruptions of the promoter sequence, large rearrangements, nonsense, frameshift, or mutations resulting in a deletion of the translation initiation signal and early stop codons, which result in no protein synthesis; (2) receptor-defective alleles, that is, transcription and missense defects that do not completely suppress the function of the protein, which has residual receptor activity; and (3) undetermined receptor activity alleles, which are splicing defects with an unknown effect on protein function [27].

Different studies have analyzed [29, 112–120] whether *LDLR* mutational class affects the prevalence of CHD risk in heterozygous FH by

**Fig. 10.5** Xanthomas in familial hypercholesteromia (FH). **a** Xanthomas on the extensor tendons of the hand in FH heterozygote. **b** and **c** tendon and tuberous xanthomas on the elbows and knees in FH homozygote. **d** Achilles tendon xanthomas in FH heterozygote. (Courtesy Prof. Francisco Carapeto)

comparing receptor-negative versus receptor-defective alleles (Table 10.3). In population with large genetic heterogeneity, most of the studies have usually found higher prevalence of xanthomas and CHD in patients with receptor-negative alleles than in those with receptor-defective alleles. However, this association was partially due to higher total and LDL cholesterol in receptor-negative subjects [119].

**Low-density Lipoprotein Concentration in FH** FH is defined by severely elevated serum LDL cholesterol concentrations from birth onwards. LDL cholesterol usually ranges from 200 to 400 mg/dL in heterozygous adults, and over 500 mg/dL in homozygous subjects from childhood. *LDLR* mutations usually present higher LDL cholesterol concentration than subjects with *APOB* or *PCSK9* mutations; and receptor-negative alleles higher LDL cholesterol than defective alleles. Those subjects with clinical diagnosis of FH, but without mutation in *LDLR, APOB,* or *PCSK9* tend to have lower LDL cholesterol than genetically well-defined FH,

$237 \pm 49$ mg/dL versus $302 \pm 69$ mg/dL, respectively [4].

## FH Diagnosis

An early diagnosis of ADH is extremely important since lipid-lowering drugs are highly effective, safe, and cost-effective in FH. The diagnosis of FH has been traditionally performed based on blood lipid values within a family, deposition of cholesterol in extravascular tissues such as tendon xanthomas or corneal arcus, and personal and family history of premature CHD.

**Homozygous FH** The diagnosis of homozygous is typically based on the presence of very high LDL cholesterol, in absence of secondary causes of hypercholesterolemia, and high LDL cholesterol in both parents. Appearance of cutaneous xanthomas, especially interdigital planar xanthomas, or tendon xanthomas prior to age 10 years is almost with high LDL cholesterol is almost pathognomonic of homozygous FH. The genetic
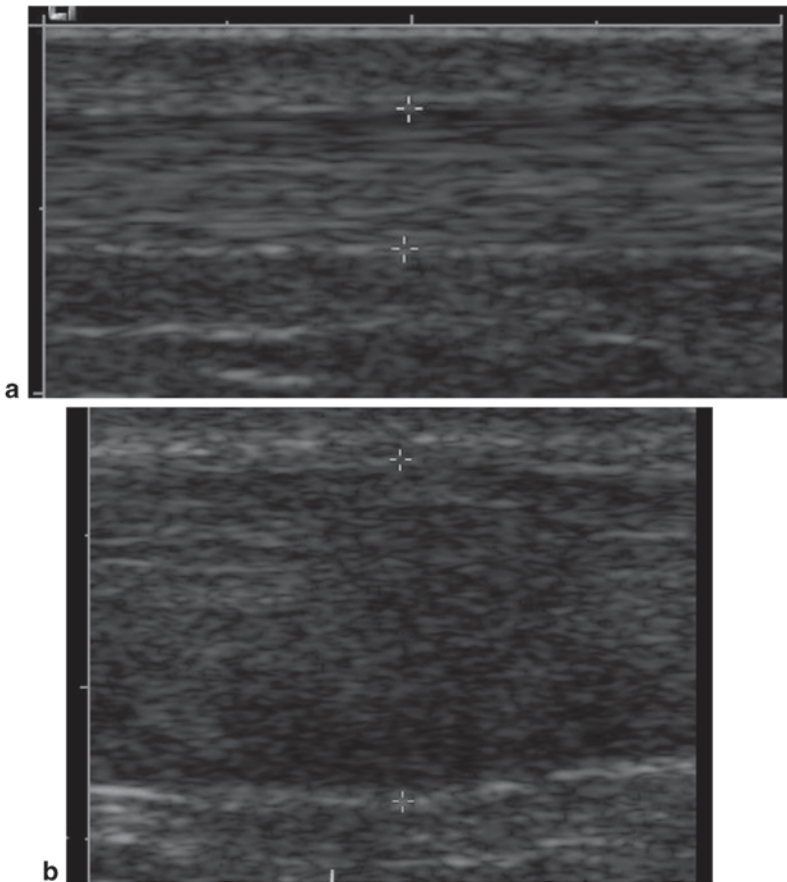
**Fig. 10.6** Achilles tendon sonographic longitudinal images. **a** normal. **b** presence of tendon xanthoma. Calipers are located in the proximal and distal borders of the Achilles tendons
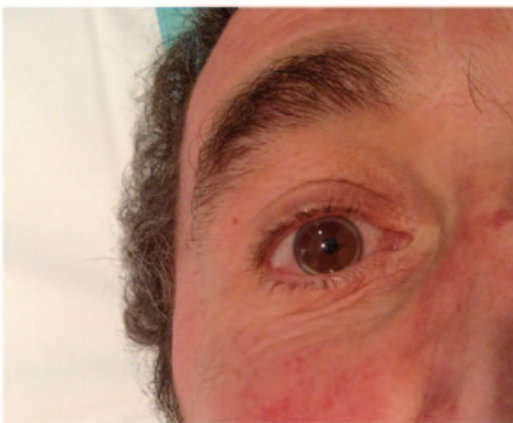


**Fig. 10.7** Corneal arcus in familial hypercholesterolemia (FH)

confirmation of two mutated *LDLR, APOB, or PCSK9* alleles and the genetic diagnosis of true homozygosity, compound heterozygosity, or double heterozygosity for FH genes is highly recommended in these subjects. The most common diagnostic criteria are presented in Table 10.4 [121].

**Heterozygous FH** Three important diagnostic criteria have been extensively used for the clinical diagnosis of FH: The Simon Broome Register Group in the UK [122], the US Make Early Diagnosis to Prevent Early Deaths (MedPed) Program [123], and the Dutch Lipid Clinic Network [124] (Table 10.5). The accuracy of these three diag-

**Table 10.3** Odds ratio (OR) for the presence of coronary heart disease (CHD) in heterozygous familial hypercholesterolemia (FH) with receptor-negative (R−) versus receptor-defective (R+) *LDLR* alleles

| Country (reference) | Year | Study sample | Total cholesterol (mg/dL) | OR (95 % confidence interval) |
|---|---|---|---|---|
| South Africa (29) | 1993 | 148 | R−: 418<br>R+: 364 | 2.6 (0.92–7.2) |
| Canada (109) | 1997 | 94 | R−: 315<br>R+: 283 | 2.7 (1.03–7.24) |
| Italy (110) | 2000 | 185 | R−: 408<br>R+: 353 | 2.6 (1.37–4.83) |
| Greece (111) | 2004 | 78 | R−: 333<br>R+: 298 | 2.6 (0.44–15.4) |
| Spain (112) | 2003 | 118 | R−: 344<br>R+: 394 | Not significant |
| The Netherlands (113) | 2005 | 645 (children) | R−: 311<br>R+: 265 | 1.22 (0.76–1.95)<br>parental CVD |
| Spain (114) | 2005 | 181 | – | 3.14 (1.00–9.87) |
| Spain (115) | 2008 | 811 | R−: 420<br>R+: 411 | 2.09 (1.04–4.21) |
| Italy (116) | 2013 | 1795 | R−: 371<br>R+: 327 | 38.1 %(R−) versus 27 % (R+),<br>$P=0.0008$ |

*OR* odds ratio, *CVD* cardiovascular disease

**Table 10.4** Diagnostic criteria for homozygous familial hypercholesterolemia (FH)

| Untreated high LDL cholesterol > 500 mg/dL after exclusion of secondary causes |
|---|
| Plus at least one: |
| Genetic confirmation of two mutated *LDLR, APOB, or PCSK9* alleles |
| Appearance of cutaneous or tendon xanthomas prior to age 10 years |
| Elevated LDL cholesterol (>200 mg/dL) and both parents consistent with heterozygous FH |
| Presence of functional FH mutations in both parents |

*LDL* low-density lipoprotein

nostic methods has not been evaluated in large, independent cohorts. Tendon xanthomas are pathognomonic of FH; however, their identification is not always easy and they are considered insensitive diagnostic markers. A high variability of xanthoma presence has been reported in FH patients [125]. Besides, tendon xanthomas may appear in patients with cerebrotendinous xanthomatosis, sitosterolemia, or dysbetalipoproteinemia. Variability in the frequency of xanthomas observed in different studies depends in part on the clinical criteria used for FH (some of them included the presence of xanthomas), as well as the methods used for the identification of xanthomas.

There are not absolutely predictive clinical criteria for the diagnosis of FH, and arbitrary criteria must be used. The criteria established by the Simon Broome Register Group from the UK for FH were based on elevated total plasma cholesterol concentration greater than 7.5 mmol/L (300 mg/dL) in the proband, together with either tendon xanthomas in the proband or in a first-degree relative or the presence of premature CHD or hypercholesterolemia in a first-degree relative [122]. The US MedPed program focuses the diagnostic criteria, principally, on high LDL cholesterol levels in the individual, and on the family history of hypercholesterolemia with evidence for a dominant transmission [123]. The presence of children with hypercholesterolemia increases the diagnostic probability. The US National Lipid Association Expert Panel on FH advises that LDL cholesterol levels > 250 mg/dL in a patient aged 30 or more >220 mg/dL for patients aged 20–29; and >190 mg/dL in patients under age 20, should prompt the clinician to strongly consider a diagnosis of FH and obtain further family informa-

**Table 10.5** Dutch Lipid Clinic Network criteria for diagnosis of heterozygous familial hypercholesterolemia (FH) in adults

| Group 1: family history | Points |
|---|---|
| First-degree relative with known premature (<55 years, men; <60 years, women) coronary heart disease (CHD) OR First-degree relative with known LDL cholesterol >95th Percentile by age and gender for country | 1 |
| First-degree relative with tendon xanthoma and/or corneal arcus OR Child(ren) <18 years with LDL cholesterol >95th percentile by age and gender for country | 2 |
| Group 2: clinical history | |
| Subject has premature (<55 years, men; <60 years, women) CHD | 2 |
| Subject has premature (<55 years, men; <60 years, women) cerebral or peripheral vascular disease | 2 |
| Group 3: physical examination | |
| Tendon xanthoma | 6 |
| Corneal arcus in a person <45 years | 4 |
| Group 4: LDL cholesterol | |
| 155–190 mg/dL (4.0–4.9 mmol/L) | 1 |
| 191–250 mg/dL (5.0–6.4 mmol/L) | 3 |
| 251–325 mg/dL (6.5–8.4 mmol/L) | 5 |
| >325 mg/dL (>8.4 mmol/L) | 8 |
| Group 5: molecular genetic testing (DNA analysis) | |
| Causative mutation shown in the *LDLR*, *APOB*, or *PCSK9* genes | 8 |

A "heterozygous" diagnosis can be made if the subject scores >8 points. A "probable FH" diagnosis can be made if the subject scores 6–8 points. A "possible FH" diagnosis can be made if the subject scores 3–5 points
*LDL* low-density lipoprotein

tion [13]. With those LDL cholesterol criteria, the sensitivity is 70% while specificity is 82% for genetically defined FH [4]. The Dutch MedPed Group described a clinical scoring system for the diagnosis of heterozygous FH patients. These criteria include personal and familial LDL cholesterol levels, history of CVD (coronary, carotid, and peripheral arteries), the presence of corneal arcus before the age of 45, and tendon xanthomas. By weighing the occurrence of these clinical signs, alone or in combination with others, a diagnostic scoring table has been constructed in the Netherlands (Table 10.5). These criteria seem to be easy to use in clinical practice and include all the clinical and laboratory features for the diagnosis of FH; and they have recently been proposed as the preferred diagnostic tool by the European Atherosclerosis Society (EAS) [14].

Molecular biology techniques have dramatically improved in recent years and more and more have become highly specific tools to improve the diagnosis of many medical conditions, including FH. Furthermore, the genetic diagno-

sis is the preferable diagnostic method in FH in most situations because it provides an unequivocal diagnosis. The National Institute for Health and Clinical Excellence (NICE) guidelines on the identification and management of FH recommend cascade screening using a combination of genetic testing and LDL cholesterol concentration measurement [126]. This approach has also been recommended by the European Atherosclerosis Society (EAS). [14].

Several methods are currently used to identify sequence changes in *LDLR*, *APOB,* and *PCSK9* genes including direct sequencing and high-throughput FH resequencing arrays [127]. A microarray for the detection of common point mutations and small deletions in the *LDLR* and *APOB* genes has been developed by our group [117]. By providing either a positive (presence of *LDLR, PCSK9,* or *APOB* mutations) or negative (absence of defects in these genes) diagnosis, this platform has allowed the genetic characterization of >8000 Spanish patients [128]. Even though the diagnosis of FH based on the detection of a functional

**Table 10.6** Indication of genetic testing in a suspected familial hypercholesterolemia (FH) proband[a]

| Subject phenotype | Indication |
|---|---|
| 1. Subjects with isolated high LDL cholesterol | |
| a. Positive personal or family history of tendon xanthomas | LDL cholesterol >160 mg/dL |
| b. Absence of personal and family history of tendon xanthomas | |
| i. Age 18–30 y | LDL cholesterol >220 mg/dL |
| ii. Age 30–39 y | LDL cholesterol >225 mg/dL, |
| iii. Age >40 y | LDL cholesterol >235 mg/dL |
| 2. Subjects with mixed hyperlipidemia (high total cholesterol and triglycerides 200–400 mg/dL) | Total cholesterol >335 mg/dL or Apolipoprotein B >185 mg/dL |

[a] Within families with clinical suspicion of FH because of vertical transmission of hypercholesterolemia and bimodal LDL cholesterol distributions in the pedigree and absence of secondary causes of hyperlipidemia (4)

*LDL* low-density lipoprotein

mutation on a causative gene is the recommended procedure in highly suspicious cases, it cannot be recommended for all cases of hypercholesterolemia. The genetic testing is still complex, expensive, and should be used as complementary to the clinical diagnosis. A group of criteria have been proposed to maximize the likelihood of genetic confirmation in subjects with clinical suspicion of FH based on age, tendon xanthomas presence, and LDL cholesterol levels (Table 10.6) [4].

## Lipid-Lowering Therapy for Heterozygous FH

Excess CHD in FH is attributable to high LDL cholesterol in this population; consequently, LDL cholesterol reduction to normal levels is a priority in the management of FH. Long-term therapy is the only way, at this time, to substantially reduce or remove the excess lifetime risk of CHD due to their genetic disorder. A healthy lifestyle is also important in the FH treatment. Lifestyle comprises a healthy diet, ideal body weight, no smoking, and adequate physical activity [4]. A healthy lifestyle provides many benefits beyond LDL cholesterol lowering, and can increase the LDL cholesterol lowering effect of drugs. Although LDL cholesterol is the fundamental CHD risk factor in FH, these subjects are very responsive to other risk factors such as smoking, which should be carefully explored and treated.

Different medical societies and expert panels have published guidelines for the management of FH, and without exception, they highly recommend aggressive LDL cholesterol lowering in all adults and less intensive treatment in children > 10 years of age (Table 10.7).

International Panel on Management of Familial Hypercholesterolemia (2004) [2]. Promoted by the Spanish Society of Arteriosclerosis, a panel of international experts proposed the first global recommendations for the diagnosis and treatment of FH: early diagnosis of the disease, a screening strategy based on finding family affected members, CHD risk stratification in heterozygous subjects according to the presence of other risk factors, early detection of atherosclerosis in preclinical phase, the establishment of three LDL cholesterol treatment goals based on baseline risk, and a therapeutic strategy based on lifestyle and pharmacological treatment with potent statins as first choice. LDL apheresis was recommended after drug treatment when LDL cholesterol is above 200 mg/dL in the presence of coronary artery disease, or above 300 mg/dL without CHD.

NICE in the UK (2008) [126] recommends a clinical diagnosis based on the criteria of Simon Broome British Register. Interestingly, a specific target LDL cholesterol target is not recommended, instead an advice to reduce LDL cholesterol by more than 50%. Baseline risk stratification before beginning the treatment was not considered. Affected children should start drug treatment after 10 years of age.

Belgian consensus for the FH treatment in children and young adults (2011) [129] focused

**Table 10.7** Summary of international guidelines for the management of familial hypercholesterolemia (FH)

| Recommendation | Country | CHD risk stratification | Recommended screening | LDL cholesterol goal | Year | Reference |
|---|---|---|---|---|---|---|
| International Panel on Management of FH | Spain | Yes (Age; gender; family history; smoking; hypertension; diabetes; LDLc>330 mg/dL; HDLc<40 mg/dL; Lp(a)>60 mg/dL) | Familial cascade | <160 mg/dL (low risk) <130 mg/dL (medium risk) <100 mg/dL (high risk or CHD) | 2004 | [2] |
| NICE | UK | NO | Familial cascade | Reduction> 50% | 2008 | [126] |
| Consensus of management of familial hypercholesterolemia in children and young adults | Belgium | Yes | Familial cascade | 10–14 years: reduction 30% >14 years:<130 mg/dL | 2011 | [129] |
| National Lipid Association | USA | Yes (Age; gender; family history; smoking; hypertension; metabolic syndrome; tendon xanthomas; LDLc>250 mg/dL; HDLc<40 mg/dL; Lp(a)>50 mg/dL) | LDLc>190 mg/dL Familial cascade | Reduction >50% (all) <160 mg/dL (low risk) <100 mg/dL (high risk) | 2011 | [130, 131] |
| European Atherosclerosis Society | Europe | No | Total chol >310 mg/dL familial cascade | <100 mg/dL <70 mg/dL (CHD, diabetes) | 2013 | [14] |

*CHD* coronary heart disease, *LDLc* low-density lipoprotein cholesterol, *HDLc* high-density lipoprotein cholesterol, *NICE* National Institute for Health and Clinical Excellence, *Lp(a)* lipoprotein (a)

on the diagnosis and treatment of children. They recommend the diagnosis and diet treatment of children from 2 years of age and consider drug treatment after 10 years of age when the LDL is >190 mg/dL or >160 mg/dL in the presence of premature CHD in the family or cardiovascular risk factors. LDL cholesterol goal of treatment is to obtain >30% LDL cholesterol reduction between 10 and 14 years, and <130 mg/dL onwards.

Lipid National Association Expert Panel on Familial Hypercholesterolemia (2011) [130, 131] from the USA published a special issue of the *Journal of Clinical Lipidology* in 2011 dedicated to FH. The documents recommend preferably clinical diagnosis based on LDL cholesterol concentrations adjusted for age, and a general population screening in subjects with LDL cholesterol levels > 190 mg/dL in adulthood, or > 160 mg/dL in children. Drug treatment is recommended when LDL cholesterol is > 190 mg/dL, with different therapeutic targets depending on individual risk factors.

Consensus Statement of the European Atherosclerosis Society (2013) [14]. This document emphasizes that FH is underdiagnosed and undertreated in the general population, defines all FH heterozygotes as high-risk patients, promotes the FH screening in subjects with plasma total cholesterol ≥310 mg/dL in adults or ≥230 mg/dL in children, premature CHD in the subject or family members, presence of tendon xanthomas in the subject or family member(s), or sudden premature cardiac death in a family member. The LDL cholesterol goals for lipid-lowering treatment are <135 mg/dL for children, <100 mg/dL for adults, and <70 mg/dL for adults with known CHD or diabetes. Therefore, high doses of potent statins, combined drug regimens usually with ezetimibe or bile acid sequestrans, and, in some cases, LDL-apheresis are required to reach such exigent targets.

Despite current maximal treatment, many heterozygous FH subjects remain with undesired high LDL cholesterol concentration. PCSK9 inhibition with two different monoclonal antibodies against PCSK9 (evolucumab and alirocumab) has been studied in two double-blind, randomized,

placebo-controlled trials in heterozygous FH insufficiently controlled with standard treatment [132, 133]. In both studies, the inhibition of PCSK yielded rapid and over 50% reductions in LDL cholesterol with good tolerability, so this therapeutic approach seems very promising in the future treatment of heterozygous FH.

## Homozygous FH Treatment

Statins, bile acid sequestrants, and ezetimibe have lesser lipid-lowering effect in homozygous FH than in heterozygous subjects, because these drugs need some LDL receptor functionality to be fully effective. However, they are remarkably safe in homozygous FH and should be tested because in some cases LDL cholesterol reductions between 20 and 40 s% can be obtained especially in LDL receptor-defective patients by increasing residual LDL receptor activity, and by inhibition of cholesterol synthesis [134].

The current treatment of choice for homozygous FH is LDL-apheresis at weekly or biweekly intervals, usually in children over the age of 7. Most homozygous FH obtain substantial reductions in LDL cholesterol, usually >50%, with periodical LDL-apheresis and is the only treatment that substantially lowers Lp(a) in these patients [121].

New drugs have been recently tested for the treatment of homozygous FH: mipomersen, lomitapide, and PCSK9 inhibitors. Mipomersen is an antisense apo B-100 mRNA recently approved for the treatment of homozygous FH. It is administered subcutaneously as a weekly injection. In a randomized, double-blind, placebo-controlled study with 34 patients, the mean LDL cholesterol reduction with this inhibitor of the apo B synthesis was 25%, although the lipid-lowering response was highly variable among individuals [135].

Lomitapide is an inhibitor of the microsomal triglyceride transfer protein (MTTP), a key protein in the assembly of apo B-containing lipoproteins in the liver and intestine. It is a highly potent lipid-lowering drug recently approved for the homozygous FH treatment in the USA and

Europe. In a single-arm, open-label study with homozygous FH, aged 18 years or older with a median dose of 40 mg a day, lomitapide reduced LDL cholesterol by 50 % after 26 weeks of treatment. Lomitapide's adverse effects included accumulation of hepatic fat. Mean hepatic fat was 1.0 % at baseline and increased to 8.6 and 8.3 % at week 56 and at week 78, respectively. The long-term consequence of this accumulation is unknown which seems to stabilize, or even decrease, with time [136].

PCSK9 inhibition has also been evaluated in homozygous FH. Alirocumab (AMG 145) a monoclonal antibody to PCSK9 demonstrates reductions of 19.2 % in LDL cholesterol in patients with defective LDL receptor activity, but no efficacy in those who were receptor negative [137].

The prognosis of intensive treated FH subjects has drastically improved in recent years reducing the CHD [138]. The key challenge in the coming years is to expand the diagnosis and treatment to this group of patients in whom cardiovascular prevention is paradigmatic and cost-effective [139].

## Autosomal Recessive Hypercholesterolemia

In 1973, a Lebanese family with an autosomal recessive form of severe hypercholesterolemia, clinically indistinguishable from FH, was described by Khachadurian et al. [140]. Afterwards, subjects with similar phenotype were identified in Sardinia [141], in subjects of Turkish and Asian-Indian origin [142], and in Japan [143], and this entity was named ARH. The causative gene, *LDLRAP1,* on chromosome 1, which encodes LDLRAP1, was identified by linkage analysis in 2001 by Garcia et al. [46]. In this gene, both homozygous and compound heterozygous mutations can be found. Most of the ARH-causing mutations are due to premature stop codons, producing no mRNA or truncated proteins [46]. The mutations identified in *LDLRAP1* gene causing ARH can be found in http://www.ucl.ac.uk/ldlr/.

LDLRAP1 is a 32-kDa and 308-amino-acid endocytic adaptor protein required for the func-

tion of LDLR in hepatocytes. LDLRAP1 protein serves as an adaptor for LDL receptor endocytosis in the liver and a deficiency in this protein results in a decrease in the LDL cholesterol catabolism [144]. The N-terminal domain of LDLRAP1 contains a phosphotyrosine-binding (PTB) domain, which binds to the internalization sequence (FDNPVY) in the cytoplasmic tail of the LDL receptor. This domain can also simultaneously interact with cell membrane phosphoinositides. Specific sequences within the C-terminal region of LDLRAP1 bind clathrin and its adaptor AP2 [145]. All these interactions together enable LDLRAP1 to function as an endocytic adaptor for the clathrin-mediated endocytosis of LDL receptor in the liver. Accordingly, LDLRAP1 has been classified as a clathrin-associated sorting protein (CLASP) [146], a group of proteins that serve as a molecular bridge between receptors and the clathrin machinery for their endocytic internalization.

ARH subjects have severely elevated plasma LDL cholesterol, tuberous and tendon xanthomata, corneal arcus, and premature atherosclerosis, with severe CHD, that make it clinically indistinguishable from FH [147]. Plasma cholesterol levels and clinical symptoms that present subjects with ARH are intermediate between those of FH heterozygotes and FH homozygotes. The age of onset of symptomatic coronary artery disease in these patients is later and tendon xanthomas tend to be large and bulky [148]. In a phenotypic comparison study between 42 ARH subjects and 42 homozygous FH subjects, Pisciotta et al. [149] reported that in ARH subjects, plasma LDL cholesterol (550±88.6 mg/dL) was lower than in receptor-negative homozygous FH (827±138 mg/dL) but similar to that found in receptor-defective homozygous FH (601±92.5 mg/dL). The risk of coronary artery disease was ninefold lower in ARH patients [150].

However, LDL receptor activity and LDL binding ability in cultured fibroblasts are normal. All *LDLRAP1* mutations characterized to date preclude the synthesis of full-length LDLRAP1, and this LDLRAP1 is required for normal LDLR function in lymphocytes and hepatocytes, but not in fibroblasts. Residual LDL receptor function in cells that do not require LDLRAP1 could explain

the reason why ARH subjects have lower plasma LDL cholesterol levels than homozygous FH patients, who have no functional LDL receptor [150].

LDL turnover studies have demonstrated that the rate of clearance of LDL from plasma is similar in subjects with ARH and in subjects with homozygous FH, and markedly reduced compared with normolipidemic controls [143], suggesting that LDLRAP1 is essential for LDL receptor-mediated uptake of LDL. Despite comparable reductions in the fractional catabolic rate of LDL, the metabolic and clinical phenotype of ARH is less severe than that of homozygous FH, as stated above [151]. ARH patients also respond to lipid-lowering drugs, as statins, with a greater reduction in plasma levels of LDL cholesterol than that observed in homozygous FH patients [152]. As the clearance rates of LDL are similarly decreased in ARH and FH, the less severe clinical phenotype in ARH points to LDL production. LDL is produced as a metabolic product of VLDL, and it has been proposed that the molecular basis for this milder phenotype is the increased removal of VLDL remnants from the circulation [153]. The increased clearance of remnant lipoproteins could contribute to the great responsiveness to statins of ARH patients [154]. Also, studies demonstrate that clearance of postprandial remnant lipoproteins is preserved in ARH in contrast to FH. This preservation of postprandial remnant particles catabolism could also contribute to the mild phenotype of ARH compared with FH [155].

## Lysosomal Acid Lipase Deficiency

Deficiency of the enzyme lysosomal acid lipase results in two distinct diseases in humans: Wolman disease and cholesteryl ester storage disease (CESD;OMIM 278000). Wolman disease is a severe lipid infiltration of the liver, spleen, and other organs early in life, causing early death in infants. Wolman disease is very rare, with an incidence of less than one in 100,000 live births. CESD is also a rare disease, around one case in 40,000 people, possibly underdiagnosed, and characterized by the accumulation of cholesterol esters in different organs of the body, especially liver. Clinically, CESD presents as a mixed hyperlipidemia in a young patient with no family history of hyperlipidemia, with hepatosplenomegaly and elevated liver enzymes. Ultrasound typically shows steatosis, but the diagnosis is usually suspected by finding a microvesicular steatosis on liver biopsy. A definitive diagnosis is made by detecting a low lysosomal acid lipase activity, or in the presence of functional mutations in the gene encoding this enzyme, LIPA [156].

The accumulation of cholesterol esters and free cholesterol reduction in the liver of these subjects leads to increased endogenous cholesterol production, and this is the believed mechanism. Evolution of these patients is to chronic liver disease, liver cirrhosis, and increased incidence of atheromatous disease [157]. Recombinant lysosomal acid lipase replacement was shown to be effective in animal models, and recently, a phase I/II clinical trial demonstrated its safety and indicated its potential metabolic efficacy [158].

## Cholesterol-7-Alpha-Hydroxylase Deficiency (OMIM 118455)

Pullinger et al. described in 2002 that mutations in the *CYP7A1* gene, encoding the enzyme cholesterol-7-alpha-hydroxylase produced hypercholesterolemia associated with heterozygosity and homozygosity, so it is considered an autosomal codominant hypercholesterolemia OMIM 118455 [159]. The enzyme cholesterol-7-alpha-hydroxylase controls the rate of conversion of cholesterol into bile acids. CYP7A1 deficiency would cause a decrease in the production of bile acids and cholesterol accumulation in the liver, causing a decrease in the expression of LDL receptors, and therefore, an increase in plasma concentration of LDL. In these patients, as liver cholesterol content is increased, statins have the desired effect of inducing the expression of LDL receptors [159].

## Summary

MHs are the most frequent inherited metabolic diseases and common causes of premature cardiovascular death and disability in most countries. They are genetically heterogeneous in which similar phenotypes may be caused by mutations in different genes, commonly *LDLR, APOB,* and *PCSK9*. However, there are some MH families in whom the responsible gene/s are unknown. Very high LDL cholesterol, familial presentation, and high prevalence of premature coronary disease are the clinical features to suspect MH. Early diagnosis of MH is very important so that therapy can be initiated as soon as possible. Combination of clinical and genetic test is the preferable diagnostic method. Familial cascade screening, once an index patient is diagnosed, is mandatory. MHs are frequently underdiagnosed and undertreated, so there is a need for a much better diagnostic screening worldwide. High doses of potent statins, combination therapy of statins with ezetimibe, or bile acid sequestrants, and, in some cases, LDL-apheresis are required to reach LDL cholesterol goals. PCSK9, apo B, and MTTP inhibitors are novel and very promising drugs that can substantially improve the treatment in the highest risk patients.

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