JIM Review

doi: 10.1111/joim.13577

Genetic and molecular architecture of familial hypercholesterolemia

Marianne Abifadel[1,2] & Catherine Boileau[1,3]

From the [1]UMR1148, Inserm, Hôpital Bichat-Claude Bernard, 46 rue Henri Huchard, F-75018 Paris, France; [2]Laboratory of Biochemistry and Molecular Therapeutics (LBTM), Faculty of Pharmacy, Pôle Technologie-Santé, Saint Joseph University of Beirut, Beirut, Lebanon; and [3]Département de Génétique, AP-HP, Hôpital Bichat-Claude Bernard, Paris, France

Abstract. Abifadel M, Boileau C. Genetic and molecular architecture of familial hypercholesterolemia. *J Intern Med.* 2023;**293**:144–165.

Atherosclerotic cardiovascular disease is the leading cause of death globally. Despite its important risk of premature atherosclerosis and cardiovascular disease, familial hypercholesterolemia (FH) is still largely underdiagnosed worldwide. It is one of the most frequently inherited diseases due to mutations, for autosomal dominant forms, in either of the *LDLR*, *APOB*, and *PCSK9* genes or possibly a few mutations in the *APOE* gene and, for the rare autosomal forms, in the *LDLRAP1* gene. The discovery of the genes implicated in the disease has largely helped to improve the diagnosis and treatment of FH from the *LDLR* by Brown and Goldstein, as well as the introduction of statins, to *PCSK9* discovery in FH by Abifadel et al., and the very rapid availability of PCSK9 inhibitors. In the last two decades, major progress has been made in clinical and genetic diagnostic tools and the therapeutic arsenal against FH. Improving prevention, diagnosis, and treatment and making them more accessible to all patients will help reduce the lifelong burden of the disease.

Keywords: APOB, APOE, familial hypercholesterolemia, gene, LDLR, mutation, PCSK9, polygenic

Introduction

Familial hypercholesterolemia (FH) is one of the most frequently inherited diseases but is still largely underdiagnosed and undertreated worldwide [1]. Indeed, the current medical practice includes questioning a possible familial disease. However, the lack of detailed questioning to build a family pedigree means that the simple current question can only reveal a possible familial aggregation (due to an underlying polygenic form of the disease with low relative risk in relatives) and not a possible familial segregation (due to the transmission of an underlying monogenic disease with a 50% risk of being a mutation carrier) with a very high risk of premature atherosclerosis and cardiovascular disease. Undertreatment of FH is also a major concern. Indeed, with the advent of statin therapy in the late 1980s and its high efficacy in lowering blood cholesterol levels of all genetic origins [2, 3] (both polygenic and monogenic), it has taken many years for the concept of "accumulated burden of low-density lipoprotein cholesterol (LDL-C) exposure over a lifetime" to be formulated [4]. With this concept, an aggressive lowering of LDL-C in mutation carriers has been recommended but is not generally performed. The major progress in the last two decades in the clinical and genetic diagnostic tools and the therapeutic arsenal against the disease has prompted many collaborative efforts of patient advocacy groups and clinicians around the world to build awareness and implement more thorough clinical recognition of FH. It was through the ground-breaking results from genetic studies at the turn of the millennium that major progress in molecular diagnosis and treatment was made possible [5].

FH is characterized by high levels of blood LDL-C, giving rise to extravascular deposits, tendon and skin xanthomas, xanthelasma, arcus cornea, and vascular deposits, causing progressive and premature atherosclerosis, coronary heart disease (CHD), and increased morbidity and mortality [6]. The genetic architecture of FH has long been held to

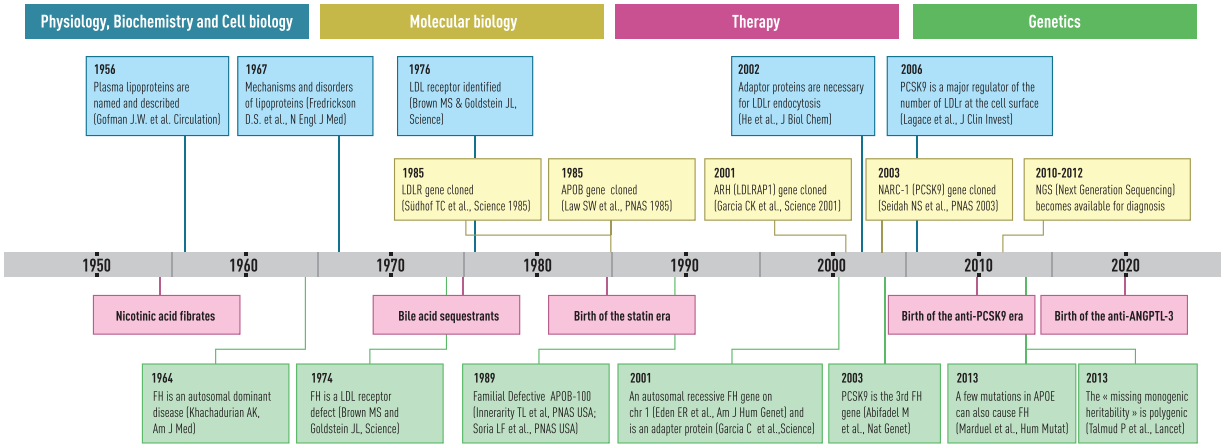

Fig. 1 *Key steps of familial hypercholesterolemia timeline.*

be monogenic. Indeed, in numerous textbooks, FH is presented as the prototypical autosomal dominant/codominant metabolic disease. However, the description of rare and clustered cases of autosomal recessive FH (or "pseudo homozygous FH") opened the way to a more varied architecture [7, 8]. This was further substantiated by the recognition of polygenic forms of the disease.

FH was originally referred to as "essential" with type IIa hyperlipoproteinemia and associated with pathogenic variants ("mutations") in the *LDLR* gene encoding the LDL transmembrane receptor (LDLr) [9]. These mutations in the *LDLR* gene were the first to be identified in the disease. As subsequent research showed the involvement of defects in other genes that also result in elevated LDL-C and the FH phenotype, the name is commonly used now for all forms of type IIa hyperlipoproteinemia with primary genetic causes, including autosomal dominant hypercholesterolemia (ADH) and autosomal recessive hypercholesterolemia (ARH).

History

Genetic studies have played a major role in the understanding and definition of the disease: (1) from Carl Muller's first report in 1938 on hereditary high blood cholesterol and its related symptoms [10], (2) through the study on the inheritance and phenotype of FH in Lebanese families by Khachadurian in 1964 [8], (3) to the identification of the LDL receptor in 1974 and the isolation of its gene in 1985 by Goldstein and Brown [9, 11], (4) the detection of the first apolipoprotein B (apo B) variant causing hypercholesterolemia and its molecular definition by Innerarity in 1984 [12] and Soria et al. in 1989 [13], and (5) the discovery of the pathogenic FH variants of *PCSK9* in 2003 by Abifadel et al. [14], with these three genes being the major genes implicated in the ADH designated by FH1, FH2, and FH3. In parallel, variants in the *LDLRAP1* gene were identified in ARH subjects in 2001 [15]. Besides diagnosis, the impact of genetic discoveries on treatment was decisive from the first statin (Merck's lovastatin) that was given FDA approval in 1986 [2, 3] to the first PCSK9 inhibitors (monoclonal antibodies) that were given approval in 2015 and showed an important reduction of LDL-C to unprecedently reached levels [16, 17] (Fig. 1).

Frequency

The prevalence of FH was long considered to be at 1:500 for heterozygous FH (HeFH) in the general population with a prevalence of 1:1,000,000 for homozygous FH (HoFH) estimated by Goldstein et al. [18] by applying the Hardy–Weinberg equation to compute genotype frequencies for the disease. The EAS Consensus Panel estimated an HeFH prevalence of 1:200 [1] and consequently 1 in 160,000–300,000 for HoFH [19]. A first pooled prevalence study of 19 published reports on over 2.4 million subjects estimated HeFH at 1:250 [20]. More recently, a meta-analysis including 11 million subjects from 104 studies reported a prevalence for HeFH of 1 in 313 in the general population [21, 22]. As expected, the frequency of both HeFH and HoFH is high in countries where founder effects

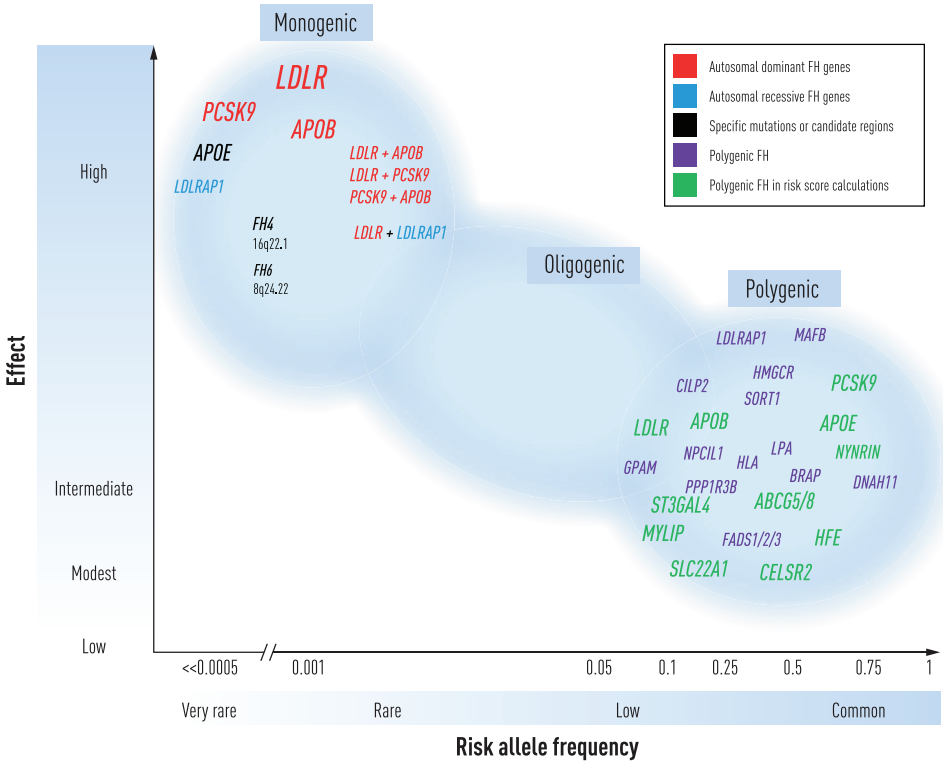

Fig. 2 *Genetic architecture and genes associated with familial hypercholesterolemia (FH). The various genetic architectures (mono, oligo, and polygenic) are defined with respect to the frequency of the alleles (specific DNA sequence) involved and the effect of the DNA variants they carry on the biological function of the encoded protein. Genes carrying pathogenic variants in autosomal dominant FH are shown in red; genes carrying pathogenic variants in autosomal recessive FH are in blue. The genes carrying variants implicated in polygenic FH are all in purple or green for those studied in polygenic risk score calculations.*

are largely documented, such as Québec, Lebanon, South Africa, and Israel [23].

Monogenic FH

The transmission mode of FH is largely autosomal dominant with a minor autosomal recessive contribution clustering in regions with high levels of consanguinity [22, 23] (Fig. 2).

Autosomal dominant hypercholesterolemia

ADH is essentially caused by pathogenic variants in either the *LDLR*, *APOB*, or *PCSK9* genes or the unexpected involvement of specific FH variants in the *APOE* gene [24] (Fig. 2). The majority of patients carry a pathogenic variant in the *LDLR* gene, which explains the confusion between the FH and ADH designations in clinical practice.

LDL receptor gene. The *LDLR* gene (OMIM-#606945) consists of 18 exons spanning 45 kilobases (kb) at 19p13.2. It encodes the 860 amino acid LDLr proprotein, which includes a 21-amino acid signal peptide [25, 26]. The LDLr proprotein is synthesized in the endoplasmic reticulum (ER), its 21 amino acid signal peptide is cleaved, and the protein is glycosylated to form the mature 160 kDa (839 amino acid) glycoprotein. This transmembrane receptor is found on the surface of most cell types and mediates the internalization of LDL into cells via receptor-mediated endocytosis [9, 25]. To date, thousands of FH variants in the *LDLR* gene have been reported in the historic locus-specific databases (UMD-LDLR [27] and University College London database [28]) as well as general databases such as HGMD and ClinVar [29]. With the advent of the more rigorous methods of variant classification (ACGS [Association for Clinical Genetic Science] and ACMG [American College of Medical

Genetics and Genomics]) [30], the number of variants classified as pathogenic or likely pathogenic (ACMG classes 4 and 5) is lower [31]. There is also an intermediate class of "variants of unknown significance" (VUS). Despite the numerous predictive tools available for variant classification, segregation analysis in families (when possible) is crucial in evaluating the pathogenicity of a variant and can provide a conclusive way to reclassify VUS variants. Interestingly, pedigree-based genetic analysis of FH, though historically highly efficient in the identification of new FH genes, was on a decline since sampling relatives at large is time consuming. However, teams are reverting to this approach to better characterize VUS variants or those with "conflicting interpretations of pathogenicity" in ClinVar [29, 32–34]. However, caution is warranted since cases of polygenic FH (not uncommon in the general population and due to the combination of genetic background and environmental factors) [25, 31] can also be found in FH families where they are phenocopies. Their inclusion in a family study will lead to an incorrect conclusion of an apparent lack of or incomplete segregation of the molecular variant in the given family. This is unavoidable but can be monitored.

The most frequent molecular alterations in the *LDLR* gene are small nucleotide variations present all over the gene, including the promoter and the 5' and 3'UTR (UnTranslated Region) regions. In ClinVar, more than 1307 pathogenic and 831 likely pathogenic variants are listed. Even though there is no particular mutation hotspot in the *LDLR* gene, some studies have reported that variants in exon 4 are usually associated with a more severe phenotype, most probably because it encodes three of the seven repeats of the "ligand-binding domain," a region that is essential for LDL binding through its apo B [25, 35]. It should be noted that common pathogenic variants are found in some ethnic/geographic groups due to founder effects, such as the p.(Cys681*) variant that accounted for 81.5% of Lebanese FH patients in a study from our group [36], five unique variants that account for 76% of the French Canadian FH population [37], G197del (FH Lithuania, now p.[Gly219del]) in Ashkenazi Jews [38], as well as specific variants in Finns [39], Afrikaners [40], and Druze [41].

Major rearrangements were the first mutations identified in the *LDLR* gene at a time when Southern blotting was commonly performed. They were the result of unequal crossing overs involving *Alu* repeats that are numerous throughout the gene. Now known as copy number variations (CNVs), they represent around 10% of causative FH variants [42] that lead to null alleles. Multiplex ligation-dependent probe amplification was used these past years to investigate these CNVs. However, they can now be easily identified by analysis of raw NGS (Next Generation Sequencing) results through bioinformatic tools, therefore greatly simplifying the analysis of the *LDLR* gene sequence with the use of a single method [42, 43]. Furthermore, with the availability of whole genome sequencing (WGS) at a lower cost, novel deep intronic variants in the *LDLR* gene are being identified in FH probands and show good segregation with the FH phenotype in families. Indeed, the effect on splicing of the (c.2140+103G>T) intronic variant was ascertained by cDNA sequencing [44], while the (c.2141-218G>A) lead to the inclusion of a pseudo-exon and appearance of a premature stop codon [45]. This proves that intronic regions of the *LDLR* should be looked a actively in mutation-negative FH patients.

The overall effect of pathogenic variants in the *LDLR* gene is either a lower number of receptors at the cell surface or the presence of functionally impaired receptors. At the cellular level, variants have been classified into five classes based on their effect on the production of LDLrs by the cell: class I, pathogenic variants affecting the synthesis of the receptor in the ER [25, 46, 47]; class II variants cause post-translation defects that block completely (class II a) or partially (class II b) LDLr trafficking to the cell membrane [25, 46–48]. Class III variants result in impaired LDL binding [25, 46, 47] while a reduced capacity for receptor-mediated endocytosis via clathrin-coated pits is observed in class IV variants [25, 46, 47] and defective LDLr recycling in class V [25, 46, 47]. This classification has limited use since functional studies are exceptionally performed and thus the vast majority of variants reported have only been characterized at the molecular level. With the advent of the more rigorous methods of variant classification, functional analyses will be performed since they enable a more definite classification of VUS. Therefore, the use of these functional classes should be more widespread in the future.

The final major genetic characteristic of FH is that it is essentially familial. Indeed, *de novo* pathogenic variants in the *LDLR* gene are exceedingly rare in our experience and that of other teams [49].

This implies that once a diagnosis of FH has been proven at the molecular level, systematic screening (or "cascade screening") of relatives needs to be vigorously performed since each has a 50% risk of carrying the same defect. Furthermore, the penetrance of FH is high (i.e., the majority of mutation carriers present with FH) and pedigree investigation often reveals undiagnosed FH-carrying relatives at risk of its life-threatening cardiovascular complications. The identification of a *de novo* mutation, although rare, should lead to the investigation of the existence either in the patient or his/her parents of a somatic and/or germline mosaicism. Although highly documented in diseases with a high mutation rate (such as Duchenne muscular dystrophy or neurofibromatosis type 1 and Marfan syndrome), this situation is very rare and has only been reported recently for FH by Rodríguez-Nóvoa et al. [50]. This original report is important since it suggests that mosaicism in FH may be underestimated. To be properly assessed, it warrants the use of an NGS technology of high depth and a good awareness of this mechanism in diagnostic laboratories. This observation could also warrant resequencing of mutation-negative FH probands who have attained high scores with the Dutch Lipid Clinic Network (DLCN) score [50].

The correlation between the variant and its impact on biological and clinical severity have been investigated in several studies. When a variant leads to a completely abnormal or absent protein (often the case with nonsense variants, altered initiation codons, frameshift variants, splicing alterations, and large deletions involving one or more exons [30]), the loss of function (LOF) of the LDL receptor is complete, and these variants are called "null" (or negative) alleles. Other nucleotide changes, usually missense changes, leading to partial LOF or altered function of the receptor, are called "defective" alleles. In HeFH cells carrying a null allele, functional characterization usually reveals a residual LDLr activity of about 50% (related to the expression of the normal *LDLR* gene allele), whereas defective alleles have greater residual activities [51, 52]. In any case, all nucleotide alterations (including synonymous, missense, and minor in-frame insertion/deletion) should be examined for a potential splicing effect [44, 53], since they could be null alleles instead of defective alleles, which could impact the severity of the phenotype. In fact, carriers of a receptor-negative/null allele that is thought to result in total loss of LDLr function have a more severe phenotype than those with defective receptor mutations, not only in terms of increased LDL-C but also in terms of a higher prevalence of tendon xanthomas, carotid atherosclerosis, and CHD [31, 54–56]). In terms of treatment response, some studies revealed that the presence of a null allele was linked to a poor response to statin therapy [31, 56–59], while others found no difference [29, 31, 60]. Recently, in HoFH patients, anti-PCSK9 monoclonal antibody evolocumab caused a significant reduction in LDL-C by 30.9% in the TESLA Part B trial in patients with *LDLR* pathogenic gene variants in both alleles, of which at least one was defective [61, 62], whereas no reduction was observed in patients homozygous for a null allele, due to the absence of functional residual LDL receptors.

APOB *gene.* The second gene implicated is *APOB* encoding apo B, one of the ligands of the LDLr. Most reported variants in the *APOB* gene are LOF variants causing hypobetalipoproteinemia. However, some missense variants are responsible for ADH [63, 64]. Indeed, Innerarity et al. reported in 1987 the first "familial defective apolipoprotein B cases" (FDB) (OMIM# 144010) [12]. This autosomal dominant genetic disease was characterized by increased LDL-C levels due to reduced hepatocyte elimination of apo B–containing particles [64]. The authors also showed that the defect was not LDLr related but the patient's LDL related. Subsequently, Soria et al. [13] identified the first and most frequent FDB pathogenic variant: p.(Arg3527Gln), originally known as APOB3500 or R3500Q. This single variant accounts for more than 95% of FDB cases [63, 65]. A few other FDB variants were reported in the following years, all located in a specific region of exon 26 of the *APOB* gene and some clustering at codon 3500 [66]. These variants—p.(Arg3527Gln), p.(Arg3527Trp), and p.(Arg3527Leu)—do not affect the binding site of apo B to the LDLr (residues 3386–3396). They affect the crucial interaction of the wild-type arginine at position 3500 with tryptophan at position 4369, near the protein's carboxy terminus. The variant at position 3500 leads to a conformational change of apo B at the surface of the LDL that alters access of the apo B binding site to its target region on the LDLr [63, 66]. The frequent p.(Arg3527Gln) FDB variant is carried by approximately 0.1% of Northern Europeans and US Caucasians. The highest frequency of carriers in Europe has been identified in German-speaking Switzerland and the highest frequency worldwide is found in the Amish population, where 12% carry the variant [67]. Thus, it is believed the variant

occurred in a common ancestor 6000–10,000 years ago in central-western Europe [68, 69]. This variant raises plasma LDL-C by approximately 60–70 mg/dl [67, 70, 71]. FDB has been associated with earlier-onset coronary artery disease and with an increased risk of ischemic heart disease [72]. FDB carriers show a higher rate of coronary artery calcification even at LDL-C levels equivalent to noncarriers and suffer myocardial infarction nearly a decade earlier than noncarriers [67, 71].

Few homozygous carriers of R3500Q have been reported to date [73–77]. Their phenotype is more severe compared with heterozygous carriers; however, the LDL-C elevation in homozygous FDB is milder compared to HoFH due to pathogenic variants in the *LDLR* gene, which can be explained by the increased clearance of certain lipoprotein subfractions. Furthermore, in both FDB heterozygotes and homozygotes, the proportion of small dense LDL particles is increased. Because small LDLs contain less cholesterol, the number of these atherogenic lipoproteins required to attain the same LDL-C concentration must be higher [78]. This could help to explain why a similar frequency of early-onset acute coronary syndrome was recently reported in a large population study in FH and FDB patients [71]. This also proves that considering LDL-C concentration levels alone might not be enough in estimating coronary artery disease risk and thus adapting treatment.

Only a few variants in the critical region of exon 26 of the *APOB* gene are proven to be causative of ADH: p.(Arg3507Trp), p.(Arg3527Gln), and p.(Trp4396Tyr) [79, 80]. They all result in defective receptor binding, most probably by altering residues that are essential for apo B–LDLr affinity binding [66, 81, 82]. Since the known FDB-causing mutations cluster within discrete regions of the *APOB* gene, classic diagnostic Sanger sequencing was long performed essentially for these regions [63, 79, 83]. With the advent of NGS either through panels or whole exome sequencing (WES) and even WGS, whole genes (or at least all their coding sequences) are being investigated and new ADH-causing variants are found in known genes [63, 84]. With these sequencing strategies, more FDB-causing variants are being reported in the *APOB* gene and not only in the original “FDB hotspot” [79]. As an illustration of this, we have recently reported the p.(Arg50Gln) variant in exon 3, a region not usually studied in ADH [63]. The same arginine at position 50 has been implicated in another FDB family carrying the p.(Arg50Trp) variant, which causes an increase in blood LDL-C and its defective hepatic uptake [85]. Furthermore, two novel *APOB* variants, p.(Arg3059Cys) and p.(Lys3394Asn), lead to a decrease in binding to the LDL receptor [63, 83]. Another study found that *APOB* p.(Arg1164Thr) and p.(Gln4494del) showed a 40% reduction in internalization in lymphocytes and HepG2 cells, similar to the *APOB* 3527 variants [63, 79]. Even though incomplete penetrance cases were reported in the families, these findings reveal that the *APOB* gene can harbor more ADH-causing variants outside of the traditionally examined regions and the entire *APOB* gene should be investigated [63, 79].

PCSK9 *gene.* The history of the discovery of PCSK9 as a major actor in FH and cholesterol metabolism highlighted the importance of genetic strategies in the discovery of new protagonists and therapeutic targets in the disease (Fig. 3). It also led to a paradigm rupture since it revealed the major regulatory role of a totally unknown actor in cholesterol homeostasis.

The recruitment and genetic analyses of families with ADH through the French Research Network for hypercholesterolemia revealed the existence of FH genes beyond the *LDLR* and *APOB* genes [86]. A linkage strategy allowed the localization of the third ADH gene (“FH3”) on the short arm of chromosome 1p32 in a multiplex French family [87]. The result was confirmed in a large family from Utah [88]. Meticulous and comprehensive studies were performed to generate a regional physical map and sequence its gene content [89, 90]. All this was performed in the pregenomic era, while the physical map of the genome was still incomplete. In parallel, other non-*LDLR*/non-*APOB* families linked to this locus were investigated. This was crucial in refining the boundaries of the chromosomal region of interest and identifying the FH3 gene [14]. Indeed, one of the genes located in the genetic interval encoded NARC (neural apoptosis regulated convertase). This protein, characterized by Seidah et al. as the ninth member of the proprotein convertase family, is highly expressed in the liver, gut, kidney, and nervous system [91]. The high expression in the liver and the genetic localization of the *NARC1* gene at 1p32 raised the hypothesis of its possible alteration in FH patients. Indeed, Abifadel et al. identified the first pathogenic variants and renamed the gene *PCSK9* [14], in agreement with the requirements of the gene nomenclature

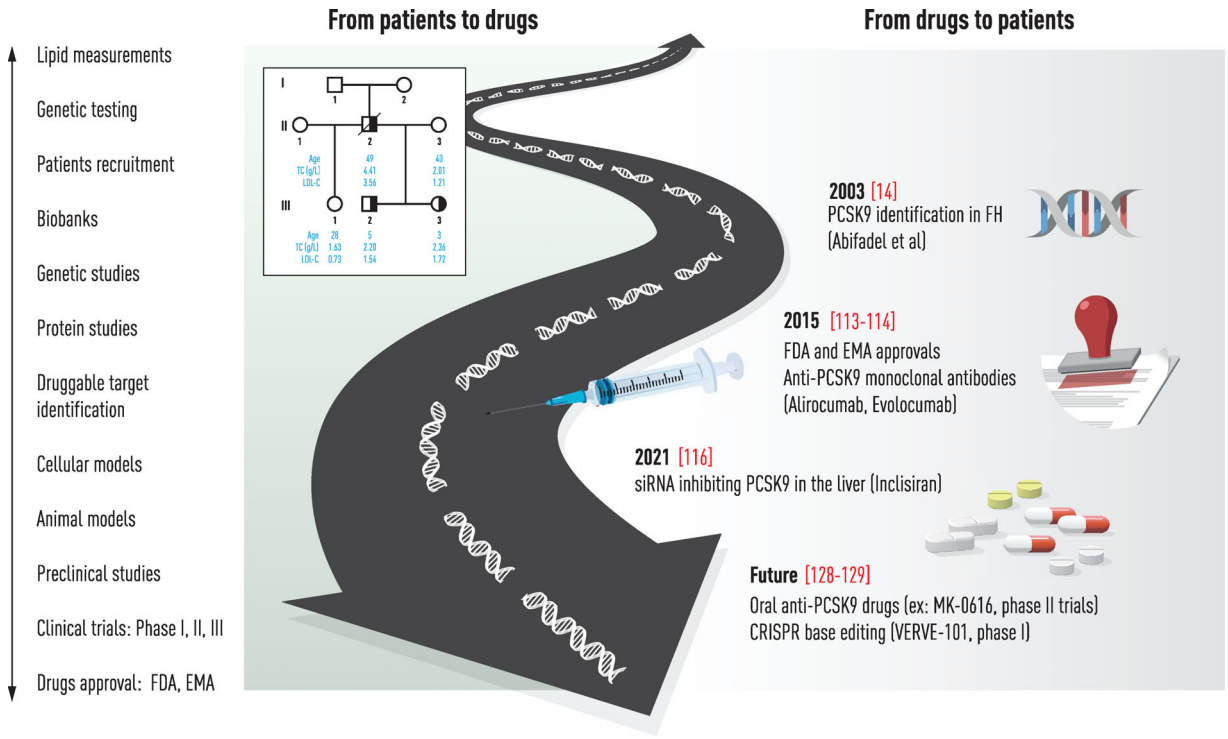

Fig. 3 *PCSK9 timeline—from discovery to targeted therapies.*

committee HUGO. This result was essential in revealing the clinical implication of *PCSK9* pathogenic variants in FH and its completely unknown implication in cholesterol homeostasis. [14].

PCSK9 *gain of function variants.* The gain of function (GOF) variant p.(Ser127Arg) was found in two large multiplex French families with several relatives presenting with hypercholesterolemia/hyper LDL-C, tendon xanthomas, myocardial infarction, and stroke. These families, originating from Nantes and Dijon, seemed to share a common ancestor as obviated by the segregation of the same haplotype (same chromosome region) with the variant in both families [14]. A nonsynonymous variant in exon 4 of *PCSK9* p.(Phe216Leu) was also identified in a family from Lille in which the proband had very high LDL-C and died from myocardial infarction at the age of 49 [14]. Soon after, a third pathogenic variant p.(Asp374Tyr) was identified in the large kindred from Utah [92] that had confirmed the original localization on chromosome 1p32 [88, 93]. This variant was later found in three Norwegian [94] and three English families [95] with, in each instance, a more severe phenotype.

PCSK9 is a secreted protein that binds (amino acids 153 and 421) to the EGF-A domain of the LDLr (position 314–355) [96]. The PCSK9-LDLr-LDL complex is internalized and traffics through the endosome where the released PCSK9-LDLr complex is targeted to the lysosome, thus reducing LDLr recycling to the cell surface. In carriers of GOF variants, as we had shown very early [97], there is a reduced number of receptors at the cell surface (notably hepatocytes) leading to LDL-C accumulation in the blood through lack of clearance [96]. The effect of GOF variants on mature PCSK9 is varied: reduction of autocatalytic cleavage and consequently the secretion of PCSK9 (with the p.(Ser127Arg) for example), increased stability/half-life of PCSK9 by decreased cleavage and inactivation by furin either completely [p. (Arg218Ser)] or partially [p.(Phe216Leu)] [98], enhanced binding affinity to LDLr receptor as compared to wild type, and its degradation in lysosome for [p.(Asp374Tyr)] [99]. The *in vivo* kinetics in two French p.(Ser127Arg) variant carriers revealed an increased (3×) production rate of apo B-100-containing lipoproteins compared with controls or *LDLR* mutated patients and a higher direct overproduction of VLDL (Very Low Density Lipoprotein)

(2×), IDL (Intermediate-Density Lipoprotein) (3×), and LDL (5×) [100]. The overproduction of VLDL lipoproteins by the liver illustrates another aspect of the role of PCSK9 in cholesterol homeostasis that remains uninvestigated.

A dozen GOF variants were initially reported between 2003 and 2008 [101] and a few others since then by our team [102, 103] and others [104]. More recently, Di Taranto et al. reported two GOF FH-causing variants: p.(Ser636Arg) and p.(Arg357Cys) [105]. It is noteworthy that our team reported in 2008 an insertion of two leucines (p.L21tri also designated p.(L15_L16ins2L)) in the leucine stretch of the signal peptide of PCSK9 that was found in two families with familial combined hyperlipidemia (FCHL) [106].

PCSK9 LOF variants. When a new gene is discovered in hypercholesterolemia, searching for variations associated with low LDL-C levels is the obvious next step since it is well known that variants in the *APOB* gene are associated with hypercholesterolemia and others with hypocholesterolemia. Thus, 2 years after the identification of the GOF variants of *PCSK9* leading to hypercholesterolemia, two frequent nonsense variants—p.(Tyr142*) and p.(Cys679*)—were identified in 2.6% of the African American subjects examined [107]. Extensive polymorphic marker analysis showed that each of these LOF variants is carried on a specific haplotype (same chromosome region), thus demonstrating that each was inherited from a common ancestor. These nonsense variants are associated with a 28% reduction in mean LDL-C level and an 88% reduction in the risk of CHD. Furthermore, another less frequent variant, [p.(Arg46Leu)], was found in Caucasian subjects from the ARIC study and was associated with a 15% reduction in LDL-C levels and a 47% reduction in the risk of CHD [108, 109]. Subsequent studies on European Caucasians identified two other hypocholesterolemic variants p.(Gly106Arg) and p. (Arg237Trp), and again notably p.(Arg46Leu). However, their overall effect on total and LDL-C blood levels is smaller than that of the two African LOFs [110].

To date, almost 1200 nucleotide variants (most of them single nucleotide variants) in *PCSK9* are found in The Genome Aggregation Database (gnomAD) [111], the largest public genomic database. Curated variants can be accessed both in ClinVar [32] and the PCSK9 Locus Specific Database [112]. In these two databases, numerous variants are listed (over 800 for the first and 308 for the second) but both are concordant for pathogenic and likely pathogenic variants numbered between 34 and 36. Therefore, among the numerous variants reported, few are relevant for molecular diagnosis of FH, contrary to the molecular landscape of the *LDLR* and *APOB* genes.

As early as the discovery that PCSK9 was a major actor in cholesterol metabolism, it was hypothesized that it represented a new target for cholesterol-lowering therapy [6]. With the determination that PCSK9 was a secreted protein and its cocrystallization bound to the LDLr, better insight was gained into the role of PCSK9 in modulating the number of LDLrs at the cell surface. This led to the design of several new anti-PCSK9 drug classes [14, 16, 62, 113–116]. The first drugs available were fully humanized anti-PCSK9 monoclonal antibodies in 2015 and recently an siRNA. Table 1 provides detailed information on these new drugs and also includes the other recent novel nonstatin therapeutic options for FH treatment, while available and future strategies to lower PCSK9 are depicted in Fig. 3 [16, 62, 113–129].

APOE *gene.* Apolipoprotein E (apo E) is a well-known protagonist of lipoprotein metabolism. Its isoforms and variants are associated with variable LDL-C levels, and different dyslipidemias, notably type III hyperlipoproteinemia (familial dysbetalipoproteinemia for apo E2 homozygotes) or FCHL. More recently, some variants in the *APOE* gene at 19q13.32 (OMIM #107741)] have been associated with ADH. First reported as responsible for sea-blue histiocytosis and FCHL, the c.500_502delTCC/[p.(Leu167del)] variant was significantly associated with FH in 14 affected members of a large French family [24]). The same variant was also identified by WES in a Canadian family of Italian origin. The proband presented with an acute myocardial infarction at age 43. He had tendinous xanthomas, xanthelasmas, and elevated levels of total and LDL cholesterol [130]. Since this report, the variant has been found in other FH families. A second *APOE* gene variant [p.(Arg163Cys) segregated with the disease at the homozygous level in a 9-year-old boy and was carried by his HeFH mother [131]. Seven other *APOE* gene variants [p.(Glu21Lys), p.(Leu46Pro), p.(Gln99Lys), p.(Pro102Arg), p.(Arg269Gly), and p.(Leu270Glu)] have been identified in FH probands with no mutation in another FH-associated gene. However, the lack of functional data and systematic

Table 1. *New strategies for lowering high cholesterol levels*

Target	Discovery related to genetic studies	Mode of action	Drug name	Approval year FDA/EMA	Dosage	Response in heterozygote FH	Response in HoFH	Side effects
PCSK9	Yes French families with FH [14] Positional cloning and sequencing	Anti-PCSK9 monoclonal antibodies	Alirocumab fully human IgG1	2015/2015	SC 75–150 mg Q2W or 300 mg Q4W	- LDL-C reduction (40%–64%) when added to a statin [16]-Lp(a) (lipoprotein(a)) reduction up to 30% [113, 114]	LDL-C reduction (20%–30%) in HoFH with defective allele [62, 115]	Well tolerated, injection site reactions, flu-like symptoms
			Evolocumab fully human IgG2	2015/2015	SC 140 mg Q2W or 420 mg Q4W			
		siRNA inhibiting PCSK9	Inclisiran	2021/2021	SC 300 mg every 3 months		LDL-C reduction (12%–37%) [116]	Injection site reactions
ATP citrate lyase	No	Inhibition of ATP citrate lyase	Bempedoic acid	2020/2021	Oral 180 mg daily Max. tolerated dose statin	Monotherapy LDL-C reduction by 30%		Hyperuricemia, gout symptoms, increased risk of tendon rupture [117]

(*Continued*)

Table 1. *Continued*

Target	Discovery related to genetic studies	Mode of action	Drug name	Approval year FDA/EMA	Dosage	Response in heterozygote FH	Response in HoFH	Side effects
ANGPTL-3	Yes GWAS and patients with *ANGPTL-3* pathogenic gene variants and deficiency [118]	Monoclonal antibody inhibiting ANGPTL-3	Evinacumab Fully human	2021/2021	IV (intra-venous)15 mg/kg every 4 weeks	No	FDA approved for HoFH reduction: LDL-C (47%); TG (triglycerides) (50%) [119]	Flu-like symptoms
MTP	Yes LOF pathogenic gene variants in the *MTTP* gene encoding MTP [120, 121]	MTP Inhibitor	Lomitapide	2012/2013	Oral 5–60 mg daily	No	Reduction: - LDL-C (50%) - Apo B (49%) - TG (45%) [122]	Hepatic steatosis, gastrointestinal disorders, impaired liver function
APOB	No	Antisense oligonucleotide targeting *APOB* mRNA	Mipomersen	2013/EMA refused	SC 200 mg weekly (160 mg in subjects <50 kg)	No	Reduction: - LDL-C (20%–50%) - Lp(a) (30%) [123]	Injection site reactions, flu-like symptoms, hepatic steatosis, impaired liver function

Note: Several new strategies for lowering high cholesterol levels are now available when the classic treatment by statins, ezetimibe, or bile acid sequestrant (cholestyramine) are insufficient to attain recommended LDL-C goals. It is noteworthy that the large majority of HoFH patients do not achieve LDL-C targets with a protocol combining lipoprotein apheresis to maximally tolerated doses of lipid-lowering medications [124].
Abbreviations: FH, familial hypercholesterolemia; GWAS, genome-wide association studies; HoFH, homozygous FH; LDL-C, low-density lipoprotein cholesterol; LOF, loss of function; SC, subcutaneously; Q2W, every 2 weeks; Q4W, every 4 weeks.

investigation of families at large does not yet allow proper classification of some of these variants [24, 130–140]. In conclusion, it is now established that *APOE* is also an FH-related gene, but only a minor contributor to the molecular pathology of FH.

The case of STAP1 and other still unknown ADH genes. The identification of FH mutations in the *PCSK9* gene triggered several genetic studies that lead to genome mapping of additional ADH genes. The STAP1(signal transducing adaptor family member 1) encoding gene was reported to be implicated in FH through localization of a new ADH gene on chromosome 4p13 and subsequent identification of a few patients with elevated LDL-C and acute myocardial infraction carrying variants of the gene, especially p.(Glu97Asp) (in the original large Dutch family), p.(Leu69Ser), p.(Pro176Ser), and p.(Thr47Ala) [141]. However, the relatively small number of carriers of variants in *STAP1*, the fact that no clear damaging variant has been reported, and the lack of association with lipid traits in a large cohort [142] provided evidence against its implication in FH. Furthermore, contrary to all genes implicated in cholesterol regulation, the *STAP1* gene is not highly expressed in hepatocytes that play a major role in cholesterol homeostasis. Recently, further results, notably extensive functional analyses in various cell models and $Stap^{-/-}$ mice, conclusively demonstrated that *STAP1* is not an FH nor an LDL-C-modulating gene and should not be considered as such for FH genetic screening [143]. This "delisting" of the *STAP1* gene [144] lessened knowledge of the underlying genetic defects in FH and it directly enlarged the number of "orphan" FH families in which the gene defect is still unknown. Beyond the story of the *STAP1* gene, other new FH genes have been located on chromosomes 16q22.1 [145] and 8q24.22 [146] but are still unknown despite extensive WES and WGS [147].

Autosomal recessive hypercholesterolemia

Besides ADH, rare cases with an autosomal recessive inheritance of FH are known. Khachadurian and Uthman reported in 1973 a Lebanese family in which four children were confirmed as HoFH despite being born to normocholesterolemic parents. Although these children had large xanthomas, their plasma cholesterol (400–470 mg/dL) was lower than that of the *LDLR*-gene-related HoFH patients (750 ± 119 mg/dL) born from hypercholesterolemic parents. They showed that in rare cases, FH might be transmitted as a recessive trait [148, 149]. The ARH gene was mapped on chromosome 1 (1p36–p35) in 2001 [15, 149]. A linkage analysis approach with homozygosity mapping in two families from Sardinia and two from Lebanon, including the original one reported by Khachadurian and Uthman, was followed by systematic sequencing of the regional genes expressed in the liver. This approach allowed Garcia et al. [15] to identify the *ARH* gene (which spans 29 kb and has nine exons). This gene, now designated *LDLRAP1* (low-density lipoprotein receptor adaptor protein 1), encodes an adaptor protein. It contains a phosphotyrosine binding domain that binds the consensus sequence NPXY present in the cytoplasmic tails of several cell-surface receptors, including the LDLr [149]. This adaptor also interacts with clathrin, the major structural component of the vesicles involved in the endocytosis of the LDLr [150, 151]. Thus, this protein is an active member of LDL-LDLr-specific endocytic pathway.

The *LDLRAP1* gene database (http://www.LOVD.nl/LDLRAP1) [152] lists 100 variants but only 15 are pathogenic and have been reported from around the world, notably in Lebanon (three mutations), Syria, Turkey (two), Iran, Pakistan, Italy (Sardinia [two] and Sicily), Spain, England (two), the United States, Mexico, Japan (two), and African-Zulu, most of them being insertions or deletions causing a frameshift and truncated protein [153]. Interestingly, approximately 0.7% of Sardinians are carriers of either of two founder mutations ARH1 for [p.(Ala145Serfs*26)] or ARH2 for [p.(Trp22Ter)] [154]. Overall, the clinical features of Sardinian ARH patients are similar to those found in HoFH with *LDLR* defective gene mutations but they have lower levels of total blood cholester and LDL-C and higher levels of HDL-C compared to HoFH homozygotes with *LDLR* negative gene mutations [54, 155]. The percentage of patients with CHD is significantly lower in ARH patients and aortic valve stenosis, aortic root disease, and ascending aorta atherosclerosis appear more rarely and later in life [156].

Compound heterozygosity and genetic modifiers of hypercholesterolemia

Identification in FH patients of more than one functional variant in genes implicated in FH has been repeatedly reported and impacts the phenotype (Fig. 2). HoFH is the severe pediatric form of the disease with cardiovascular complications appearing

before the age of 20 years. It is usually due to the same mutation inherited from both parents, especially in consanguineous families. However, the HoFH clinical form is also associated either with compound heterozygosity or double heterozygosity [157–159]. In compound heterozygosity, both alleles of the same gene carry a different pathogenic variant (this is often found in HoFH associated with variants in the *LDLR* gene) whereas in double or combined heterozygosity, a patient carries heterozygous variants in two FH genes. Thus, double heterozygosity has been reported in several carriers of variants in both *LDLR* and *PCSK9* genes, *LDLR* and *APOB*, or *APOB* and *PCSK9*, as well as *LDLR* and *LDLRAP1* [160].

A double heterozygous patient displaying a severe phenotype associated with a pathogenic variant in *PCSK9* [p.(Arg469Trp) and the c.1209delC p.(Met412fs*) in the *LDLR* was identified in 2005 [161]. Subsequently, other double heterozygotes carrying variants in the *LDLR* and *PCSK9* genes were reported [162]. Elbitar et al. also reported a patient suffering from severe FH who carried hypercholesterolemic variants both in *PCSK9* [p.(Arg96Cys) and *APOB* [p.(Arg3396Thr)] [63]. The severity of the phenotype and the elevation of LDL-C levels before treatment are frequently aggravated by the second mutation in other genes [159, 163]. In other instances, the coexistence of pathogenic variants may lead to another phenotype such as in a patient with type III hyperlipoproteinemia due to the combined action of the p.(Arg496Gln) variant in *PCSK9* and homozygosity for the apo E-2 allele [164]. Interestingly, patients with *LDLR* and *PCSK9* gene variants were at higher risk with worse atherogenic lipid profiles and cardiovascular outcomes. Lifelong exposure to high LDL-C levels significantly contributes to CHD, which usually occurs between 30 and 40 years in HeFH patients, but before 20 years in HoFH [159, 165]. Event curves for double heterozygotes (*LDLR*/*APOB* and *LDLR*/*PCSK9*) represent an intermediate mean and wide range of LDL-C values compared with the curves of true *APOB* and *PCSK9* homozygote curves [159]. Individuals who are double heterozygotes have characteristics that are more severe than those who have HeFH [166]. The combined effect of the heterozygous FH variants is varied—in some cases, the compound heterozygous patients have the same or a worse phenotype than patients with HoFH [167]. In other instances, the phenotype can be mild or normal if one of the variants decreases LDL-C levels. This is illustrated by the recent report of a healthy woman with normal to moderate cholesterol increase, despite being the offspring of a patient with HoFH [168]. The molecular analysis showed the presence of the p.(Arg46Leu) LOF variant in PCSK9, with the p.(Asp301Gly) pathogenic variant in LDLR. The effect of the PCSK9 variant (increased recycling of the functional LDLr) compensates for the pathogenic effect of the mutant LDLr.

Beyond the coexistence in double heterozygotes of pathogenic variants in two genes, other combinations are now more frequently reported as WES and WGS become available and less costly. Indeed, the effect of combinations of pathogenic variants and more frequent variants affecting blood cholesterol levels on the severity of the disease is obviated in genotype-phenotype studies. In this way, Abifadel et al. reported that in a Lebanese group of patients carrying the same pathogenic variant [p.(Cys681Ter), known as the Lebanese allele] in the *LDLR* gene, the common p.(Leu21dup) variant of *PCSK9*, known to be associated with lower LDL-C levels in general populations, is also associated with a reduction of LDL-C levels in the FH Lebanese patients [36]. This was one of the first reports of an “FH modifier”. New genes/variants underlying phenotypic FH have been identified, such as *ANGPTL3* for familial hypobetalipoproteinemia 2 (FHLB2; OMIM605019), *CH25H* (which encodes cholesterol 25-hydroxylase), and *INSIG2* (a transcription regulator in the same metabolic pathway) [169–172]. These newly identified genes have been shown to modulate the phenotype of traditional HeFH as well [159, 170]. Identification of modifiers of FH is important since it provides insight into disease prognosis in FH patients and could participate in personalized medicine, but also identify candidate proteins to target to fight disease complications. It is noteworthy that the identification that *ANGPTL3* gene variants are responsible for familial hypolipidemia [173] opened the way to ANGPTL3-targeting therapy that, in turn, was shown to be highly effective in decreasing LDL-C in HoFH patients (with negative or defective LDL receptors) [174]. Thus, ANGPTL3 targeting is part of the new arsenal of drugs for the treatment of FH (Table 1).

Polygenic FH

A large number of genome-wide association studies (GWAS) have been conducted and helped in deciphering the impact of frequent and rare

genetic variants in numerous common diseases, including dyslipidemias. Many genetic variants associated with LDL-C levels have been revealed especially through large-scale studies including populations from multiple cohorts and ethnicity (notably UK Biobank or Million Veterans Program) [175, 176]. The most recent GWAS meta-analysis performed on over 1.65 million individuals belonging to five major genetic ancestry groups identified 941 lipid-associated genomic loci [177]. All these studies underscore the complexity of the genetic architecture underlying common dyslipidemias, notably hypercholesterolemia.

Through the years and in the hands of numerous laboratories, the diagnosis of FH using standard techniques allowed the detection of a pathogenic variant in only 60%–80% of patients with definite FH and only 20%–30% of patients with possible FH, defined using the DLCN score [178–181]. In this context, Talmud et al. hypothesized in 2013 [181] that this "missing monogenic heritability" could be explained by the existence of FH patients presenting with a polygenic form of hyper-LDL-C and that the disease could be the consequence of an accumulation of frequent LDL-C-raising alleles with small effects individually (Fig. 2). They predicted that in individuals carrying several LDL-C-raising SNPs (Small Nucleotide Polymorphisms), LDL-C concentrations would exceed the diagnostic LDL-C threshold of 4.9 mmol/L used in the diagnosis of FH. Based on this hypothesis, a weighted polygene risk score (PRS) of 12 frequent LDL-C-raising alleles (chosen among those identified by Teslovitch et al. in 2010) [182] (Fig. 2) was studied in FH subjects who had a clinical diagnosis of FH with (mutation positive) or without (mutation negative) an identified FH-causing variant. The gene scores of FH subjects were overall higher than those of healthy individuals of European ancestry. Furthermore, the scores of the FH mutation-negative group were higher than the FH mutation-positive group. Subsequently, a reduced six-marker PRS performing as well was proposed [183]. These results confirmed the hypothesis that a polygenic architecture could explain severe hypercholesterolemia in an important number of FH patients for whom no single large effect variant or mutation is identified.

Effect of polygenic background on LDL-C levels in FH mutation carriers. Early on in the study of FH, the existence of variability of the disease (expressed both in terms of LDL-C levels and cardiovascular complications) was noted. The variability is observed both between families and between patients within a family. In the first case, this is partly attributed to underlying genetic heterogeneity with the involvement of the different disease genes (listed in the paragraphs above) as well as different pathogenic variants with different functional effects in a given disease gene (e.g., LDLr-negative vs. LDLr-defective situations). Explaining variability between affected members of a single family is not as straightforward. An attractive hypothesis is an effect on disease expression of an additional polygenic contribution that could either attenuate or aggravate the effect of the FH-initiating mutation. This polygenic contribution could be investigated (through the use of a PRS) and is expected to explain more highly elevated LDL-C levels in some carriers of an identical pathogenic variant in a given FH gene. It could also explain the "phenocopies" (affected relatives who do not carry the familial pathogenic variant) observed in FH families. To address this hypothesis, Ghaleb et al. calculated the weighted six-marker PRS in members from five French FH families where a mutation was identified (FH/M+) as well as some phenocopies (FH/M−) [184]. They showed that the PRS can be used as a marker of the severity of hypercholesterolemia explaining the variability of the FH phenotype observed among patients in the same family. However, it does not allow for distinguishing of phenocopies within FH families nor distinguishing of clinically affected and unaffected individuals from the same family because FH patients did not exhibit higher PRS than controls [184].

In general, conflicting results have been reported on the effectiveness of the use of a PRS in FH. On the one hand, different PRSs have been described and reported to explain high cholesterol levels or clinically diagnosed mutation-negative FH cases [185]. On the other hand, studies could not confirm the use of PRS as a reliable tool to diagnose polygenic hypercholesterolemia in these patients. Sjouke et al. [186] and Paquette et al. [187] showed that the PRS_{LDL} is not associated with risk of coronary artery disease or cardiovascular disease [187] despite an association with lipid trait. In conclusion, it is presently unclear what a PRS will add to the array of markers already used for clinical diagnosis of FH [188].

Impact of polygenic background on the risk of cardiovascular disease in FH. For any given LDL-C, FH mutation carriers are at substantially

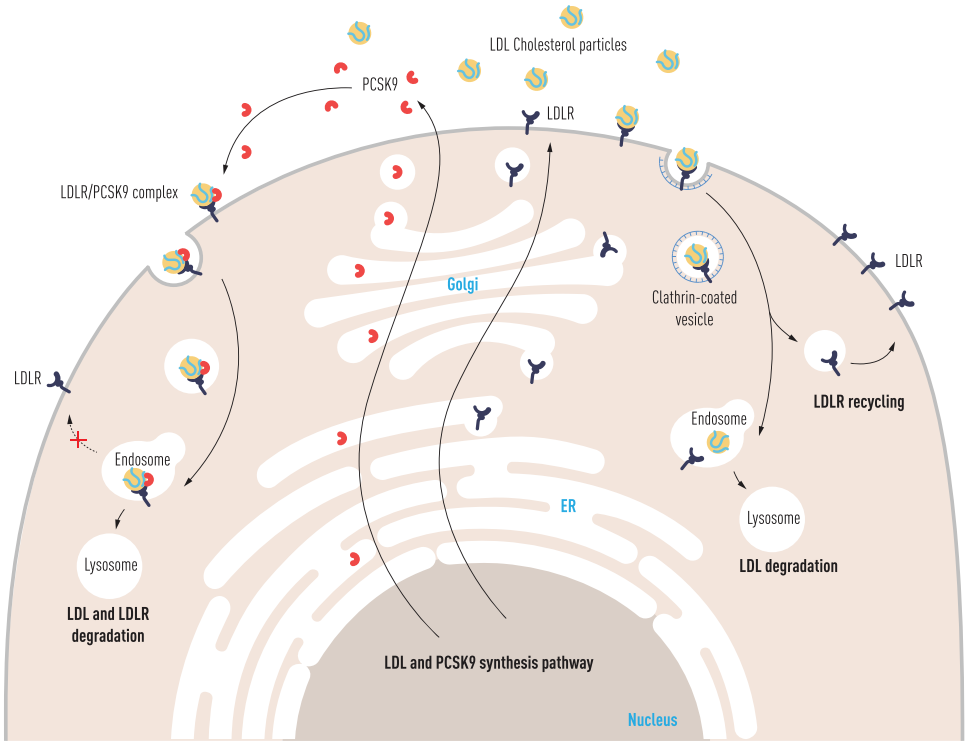

Fig. 4 *Cellular localization of the various protagonists encoded by the genes mutated in familial hypercholesterolemia.*

increased risk for atherosclerotic cardiovascular disease (ASCVD) [55].

Furthermore, even between individuals with monogenic FH, an interindividual variability in LDL-C levels and risk of ASCVD exists; this variability could be caused by an underlying polygenic contribution. Trinder et al. constructed weighted LDL-C PRSs ranging from 223 SNPS (with a significant predictive ability using only 75 of them) [185] to a more adaptable 28 SNP score [189]. With these studies [189, 190], he conclusively demonstrated that the variability of both LDL-C levels and ASCVD in subjects with monogenic FH could be explained by an underlying polygenic architecture. Furthermore, when compared to polygenic FH and hypercholesterolemia with an unknown genetic cause, the greatest risk of CHD was associated with monogenic FH. This was also found in studies for levels of carotid intimamedia thickening and coronary calcification, which were both higher in monogenic FH patients compared to polygenic patients with identical LDL-C levels [191, 192]. Further research is necessary to reach a consensus on which combination(s) of SNPs should be used, how should the PRS be expressed, and what are the cut-offs for the various risk categories. Furthermore, although the clinical and molecular definition of mutation-positive FH is straightforward, the definitions of mutation-negative FH and polygenic hypercholesterolemia need to be carefully clarified and possibly combined.

Importance of genetic testing in FH

FH is a classic inherited disease in which genetic testing should be performed for all index cases/probands to identify the disease-causing DNA variant. Therefore, genetic testing should be offered in sequential steps ("cascade screening") to first-degree relatives and extended to second- and third-degree relatives [193]. In 2013, The European Atherosclerosis Society critically evaluated the extent to which FH is underdiagnosed and undertreated worldwide and issued a consensus statement recommending molecular genetic testing in patients with definite or probable FH according to the DLCN score. This was followed by joint [194] and separate recommendations from the European Society of Cardiology [195] and the

American College of Cardiology, by institutions in the UK (NICE), France (NSFA) [196], and the United States (CDC) as well as patient advocacy groups (Family Heart Foundation) [34, 197, 198].

It is noteworthy that genetic testing has the potential to provide a definitive diagnosis; guide optimal management, genetic counseling, and prevention; initiate and adapt the most effective treatment quickly and eventually a more aggressive and earlier strategy; help in the management of the disease; and prevent its complications [199].

Besides the clinical impact of FH, the economic burden of hypercholesterolemia also highlights the importance of FH diagnosis to prevent and treat the disease and avoid its complications. This should be generalized to all countries, including those with low income, that need to be helped with the implementation of these procedures. Furthermore, with the spread of machine-learning technologies and digital technology, the identification of probands could be facilitated through the exploration of electronic health records and the creation of clinical alerts and would allow an improvement of diagnostic strategies and classify FH patients with high specificity in pediatric datasets [200] and adults [201]. The incorporation of universal screening of FH can be improved as well [202, 203].

In conclusion, the field of FH has been rejuvenated with the results of genetic studies that have revealed that the complexity of cholesterol homeostasis in humans was still incompletely unraveled and thus needed further investigation (Fig. 4). These genetic studies, in combination with new therapeutic designs (fully humanized monoclonal antibodies, siRNA), have also allowed the development of the first new class of highly efficient cholesterol-lowering drugs since statins. It is now necessary for the scientific and medical communities as well as patient advocacy groups to actively lobby for policymakers to fund both research in the field as well as FH diagnosis, prevention, and treatment to make them accessible to all patients. This should largely reduce the burden of CHD in all countries.

Acknowledgments

C.B. gratefully acknowledges the support of the Lefoulon-Delalande Foundation. M.A. acknowledges support from Université Saint Joseph-Beyrouth.

Conflict of interest

No conflict of interest was declared.

References

1 Nordestgaard BG, Chapman MJ, Humphries SE, Ginsberg HN, Masana L, Descamps OS, et al. Familial hypercholesterolaemia is underdiagnosed and undertreated in the general population: guidance for clinicians to prevent coronary heart disease: consensus statement of the European Atherosclerosis Society. *Eur Heart J.* 2013;**34**:3478–90a.
2 Endo A. A historical perspective on the discovery of statins. *Proc Jpn Acad Ser B Phys Biol Sci.* 2010;**86**:484–93.
3 Endo A. A gift from nature: the birth of the statins. *Nat Med.* 2008;**14**:1050–2.
4 Brown MS, Goldstein JL. Biomedicine. Lowering LDL—not only how low, but how long? *Science.* 2006;**311**:1721–3.
5 Shapiro MD, Tavori H, Fazio S. PCSK9: from basic science discoveries to clinical trials. *Circ Res.* 2018;**122**:1420–38.
6 Goldstein JL, Brown MS. Familial hypercholesterolemia: pathogenesis of a receptor disease. *Johns Hopkins Med J.* 1978;**143**:8–16.
7 Morganroth J, Levy RI, McMahon AE, Gotto AM. Pseudohomozygous type II hyperlipoproteinemia. *J Pediatr.* 1974;**85**:639–43.
8 Khachadurian AK. The inheritance of essential familial hypercholesterolemia. *Am J Med.* 1964;**37**:402–7.
9 Goldstein JL, Brown MS. The LDL receptor and the regulation of cellular cholesterol metabolism. *J Cell Sci Suppl.* 1985;**3**:131–7.
10 Muller C. Angina pectoris in hereditary xanthomatosis. *Nutrition Reviews.* 1987;**45**:113–5.
11 Goldstein JL, Brown MS. Binding and degradation of low density lipoproteins by cultured human fibroblasts. Comparison of cells from a normal subject and from a patient with homozygous familial hypercholesterolemia. *J Biol Chem.* 1974;**249**:5153–62.
12 Innerarity TL, Weisgraber KH, Arnold KS, Mahley RW, Krauss RM, Vega GL, et al. Familial defective apolipoprotein B-100: low density lipoproteins with abnormal receptor binding. *Proc Natl Acad Sci USA.* 1987;**84**:6919–23.
13 Soria LF, Ludwig EH, Clarke HR, Vega GL, Grundy SM, McCarthy BJ. Association between a specific apolipoprotein B mutation and familial defective apolipoprotein B-100. *Proc Natl Acad Sci U S A.* 1989;**86**:587–91.
14 Abifadel M, Varret M, Rabès J-P, Allard D, Ouguerram K, Devillers M, et al. Mutations in PCSK9 cause autosomal dominant hypercholesterolemia. *Nat Genet.* 2003;**34**:154–6.
15 Garcia CK, Wilund K, Arca M, Zuliani G, Fellin R, Maioli M, et al. Autosomal recessive hypercholesterolemia caused by mutations in a putative LDL receptor adaptor protein. *Science* 2001;**292**:1394–8.
16 El Khoury P, Elbitar S, Ghaleb Y, Khalil YA, Varret M, Boileau C, et al. PCSK9 mutations in familial hypercholesterolemia: from a groundbreaking discovery to anti-PCSK9 therapies. *Curr Atheroscler Rep.* 2017;**19**:49.
17 Everett BM, Smith RJ, Hiatt WR. Reducing LDL with PCSK9 inhibitors—the clinical benefit of lipid drugs. *N Engl J Med.* 2015;**373**:1588–91.
18 Goldstein J, Hobbs H, Brown M. The metabolic and molecular bases of inherited disease. Scriver CR, Beaudet AL,

Sly WS, Valle D, editors. *Familial hypercholesterolemia*. New York: McGraw-Hill; 2001. p. 2863–913.
19 Cuchel M, Bruckert E, Ginsberg HN, Raal FJ, Santos RD, Hegele RA, et al. Homozygous familial hypercholesterolaemia: new insights and guidance for clinicians to improve detection and clinical management. A position paper from the Consensus Panel on Familial Hypercholesterolaemia of the European Atherosclerosis Society. *Eur Heart J.* 2014;**35**:2146–57.
20 Akioyamen LE, Genest J, Shan SD, Reel RL, Albaum JM, Chu A, et al. Estimating the prevalence of heterozygous familial hypercholesterolaemia: a systematic review and meta-analysis. *BMJ Open.* 2017;**7**:e016461.
21 Kastelein JJP, Reeskamp LF, Hovingh GK. Familial hypercholesterolemia: the most common monogenic disorder in humans. *J Am Coll Cardiol.* 2020;**75**:2567–9.
22 Beheshti SO, Madsen CM, Varbo A, Nordestgaard BG. Worldwide prevalence of familial hypercholesterolemia: meta-analyses of 11 million Subjects. *J Am Coll Cardiol.* 2020;**75**:2553–66.
23 Austin MA, Hutter CM, Zimmern RL, Humphries SE. Genetic causes of monogenic heterozygous familial hypercholesterolemia: a HuGE prevalence review. *Am J Epidemiol.* 2004;**160**:407–20.
24 Marduel M, Ouguerram K, Serre V, Bonnefont-Rousselot D, Marques-Pinheiro A, Erik Berge K, et al. Description of a large family with autosomal dominant hypercholesterolemia associated with the APOE p.Leu167del mutation. *Hum Mutat.* 2013;**34**:83–7.
25 Brautbar A, Leary E, Rasmussen K, Wilson DP, Steiner RD, Virani S. Genetics of familial hypercholesterolemia. *Curr Atheroscler Rep.* 2015;**17**:491.
26 Leigh SEA, Foster AH, Whittall RA, Hubbart CS, Humphries SE. Update and analysis of the University College London low density lipoprotein receptor familial hypercholesterolemia database. *Ann Hum Genet.* 2008;**72**:485–98.
27 Villéger L, Abifadel M, Allard D, Rabès J-P, Thiart R, Kotze MJ, et al. The UMD-LDLR database: additions to the software and 490 new entries to the database. *Hum Mutat.* 2002;**20**:81–7.
28 Usifo E, Leigh SEA, Whittall RA, Lench N, Taylor A, Yeats C, et al. Low-density lipoprotein receptor gene familial hypercholesterolemia variant database: update and pathological assessment. *Ann Hum Genet.* 2012;**76**:387–401.
29 Mata N, Alonso R, Badimón L, Padró T, Fuentes F, Muñiz O, et al. Clinical characteristics and evaluation of LDL-cholesterol treatment of the Spanish Familial Hypercholesterolemia Longitudinal Cohort Study (SAFEHEART). *Lipids Health Dis.* 2011;**10**:94.
30 Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med.* 2015;**17**:405–24.
31 Di Taranto MD, Giacobbe C, Fortunato G. Familial hypercholesterolemia: a complex genetic disease with variable phenotypes. *Eur J Med Genet.* 2020;**63**:103831.
32 Landrum MJ, Lee JM, Riley GR, Jang W, Rubinstein WS, Church DM, et al. ClinVar: public archive of relationships among sequence variation and human phenotype. *Nucleic Acids Res.* 2014;**42**:D980–5.
33 Iacocca MA, Chora JR, Carrié A, Freiberger T, Leigh SE, Defesche JC, et al. ClinVar database of global familial hypercholesterolemia-associated DNA variants. *Hum Mutat.* 2018;**39**:1631–40.
34 Abifadel MS, Rabès J-PH, Boileau CR. Genetic testing in familial hypercholesterolemia: strengthening the tools, reinforcing efforts, and diagnosis. *JACC Basic Transl Sci.* 2021;**6**:831–3.
35 Gudnason V, Day IN, Humphries SE. Effect on plasma lipid levels of different classes of mutations in the low-density lipoprotein receptor gene in patients with familial hypercholesterolemia. *Arterioscler Thromb.* 1994;**14**:1717–22.
36 Abifadel M, Rabès J-P, Jambart S, Halaby G, Gannagé-Yared M-H, Sarkis A, et al. The molecular basis of familial hypercholesterolemia in Lebanon: spectrum of LDLR mutations and role of PCSK9 as a modifier gene. *Hum Mutat.* 2009;**30**:E682–91.
37 Davignon J, Roy M. Familial hypercholesterolemia in French–Canadians: taking advantage of the presence of a "founder effect". *Am J Cardiol.* 1993;**72**:6D–10D.
38 Durst R, Colombo R, Shpitzen S, Avi LB, Friedlander Y, Wexler R, et al. Recent origin and spread of a common Lithuanian mutation, G197del LDLR, causing familial hypercholesterolemia: positive selection is not always necessary to account for disease incidence among Ashkenazi Jews. *Am J Hum Genet.* 2001;**68**:1172–88.
39 Koivisto UM, Turtola H, Aalto-Setälä K, Top B, Frants RR, Kovanen PT, et al. The familial hypercholesterolemia (FH)—North Karelia mutation of the low density lipoprotein receptor gene deletes seven nucleotides of exon 6 and is a common cause of FH in Finland. *J Clin Invest.* 1992;**90**:219–28.
40 Kotze MJ, De Villiers WJ, Steyn K, Kriek JA, Marais AD, Langenhoven E, et al. Phenotypic variation among familial hypercholesterolemics heterozygous for either one of two Afrikaner founder LDL receptor mutations. *Arterioscler Thromb.* 1993;**13**:1460–8.
41 Landsberger D, Meiner V, Reshef A, Levy Y, van der Westhuyzen DR, Coetzee GA, et al. A nonsense mutation in the LDL receptor gene leads to familial hypercholesterolemia in the Druze sect. *Am J Hum Genet.* 1992;**50**:427–33.
42 Berberich AJ, Hegele RA. The role of genetic testing in dyslipidaemia. *Pathology.* 2019;**51**:184–92.
43 Iacocca MA, Wang J, Dron JS, Robinson JF, Mcintyre AD, Cao H, et al. Use of next-generation sequencing to detect LDLR gene copy number variation in familial hypercholesterolemia. *J Lipid Res.* 2017;**58**:2202–9.
44 Reeskamp LF, Hartgers ML, Peter J, Dallinga-Thie GM, Zuurbier L, Defesche JC, et al. A deep intronic variant in LDLR in familial hypercholesterolemia. *Circ Genom Precis Med.* 2018;**11**:e002385.
45 Reeskamp LF, Balvers M, Peter J, Van De Kerkhof L, Klaaijsen LN, Motazacker MM, et al. Intronic variant screening with targeted next-generation sequencing reveals first pseudoexon in LDLR in familial hypercholesterolemia. *Atherosclerosis.* 2021;**321**:14–20.
46 Hopkins PN, Toth PP, Ballantyne CM, Rader DJ, National Lipid Association Expert Panel on Familial Hypercholesterolemia. Familial hypercholesterolemias: prevalence, genetics, diagnosis and screening recommendations from

the National Lipid Association Expert Panel on Familial Hypercholesterolemia. *J Clin Lipidol.* 2011;**5**:S9–17.
47 Hobbs HH, Russell DW, Brown MS, Goldstein JL. The LDL receptor locus in familial hypercholesterolemia: mutational analysis of a membrane protein. *Annu Rev Genet.* 1990;**24**:133–70.
48 Hobbs HH, Brown MS, Goldstein JL. Molecular genetics of the LDL receptor gene in familial hypercholesterolemia. *Hum Mutat.* 1992;**1**:445–66.
49 Pisciotta L, Cantafora A, De Stefano F, Langheim S, Calandra S, Bertolini S. A "de novo" mutation of the LDL-receptor gene as the cause of familial hypercholesterolemia. *Biochim Biophys Acta.* 2002;**1587**:7–11.
50 Rodríguez-Nóvoa S, Rodríguez-Jiménez C, Alonso C, Rodriguez-Laguna L, Gordo G, Martinez-Glez V, et al. Familial hypercholesterolemia: a single-nucleotide variant (SNV) in mosaic at the low density lipoprotein receptor (LDLR). *Atherosclerosis.* 2020;**311**:37–43.
51 Romano M, Di Taranto MD, D'Agostino MN, Marotta G, Gentile M, Abate G, et al. Identification and functional characterization of LDLR mutations in familial hypercholesterolemia patients from Southern Italy. *Atherosclerosis.* 2010;**210**:493–6.
52 Romano M, Di Taranto MD, Mirabelli P, D'Agostino MN, Iannuzzi A, Marotta G, et al. An improved method on stimulated T-lymphocytes to functionally characterize novel and known LDLR mutations. *J Lipid Res.* 2011;**52**:2095–100.
53 Ho CKM, Musa FR, Bell C, Walker SW. LDLR gene synonymous mutation c.1813C>T results in mRNA splicing variation in a kindred with familial hypercholesterolaemia. *Ann Clin Biochem.* 2015;**52**:680–4.
54 Bertolini S, Pisciotta L, Rabacchi C, Cefalā AB, Noto D, Fasano T, et al. Spectrum of mutations and phenotypic expression in patients with autosomal dominant hypercholesterolemia identified in Italy. *Atherosclerosis.* 2013;**227**:342–8.
55 Khera AV, Won H-H, Peloso GM, Lawson KS, Bartz TM, Deng X, et al. Diagnostic yield and clinical utility of sequencing familial hypercholesterolemia genes in patients with severe hypercholesterolemia. *J Am Coll Cardiol.* 2016;**67**:2578–89.
56 Rubba P, Gentile M, Marotta G, Iannuzzi A, Sodano M, De Simone B, et al. Causative mutations and premature cardiovascular disease in patients with heterozygous familial hypercholesterolaemia. *Eur J Prev Cardiol.* 2017;**24**:1051–9.
57 Chaves FJ, Real JT, García-García AB, Civera M, Armengod MAE, Ascaso JF, et al. Genetic diagnosis of familial hypercholesterolemia in a South European outbreed population: influence of low-density lipoprotein (LDL) receptor gene mutations on treatment response to simvastatin in total, LDL, and high-density lipoprotein cholesterol. *J Clin Endocrinol Metab.* 2001;**86**:4926–32.
58 Santos PCJL, Morgan AC, Jannes CE, Turolla L, Krieger JE, Santos RD, et al. Presence and type of low density lipoprotein receptor (LDLR) mutation influences the lipid profile and response to lipid-lowering therapy in Brazilian patients with heterozygous familial hypercholesterolemia. *Atherosclerosis.* 2014;**233**:206–10.
59 Viladés Medel D, Leta Petracca R, Carreras Costa F, Cardona Olle M, Barros Membrilla A, Hidalgo Perez JA, et al. Coronary computed tomographic angiographic findings in asymptomatic patients with heterozygous familial hypercholesterolemia and null allele low-density lipoprotein receptor mutations. *Am J Cardiol.* 2013;**111**:955–61.
60 Choumerianou DM, Dedoussis GVZ. Familial hypercholesterolemia and response to statin therapy according to LDLR genetic background. *Clin Chem Lab Med.* 2005;**43**:793–801.
61 Najam O, Ray KK. Familial hypercholesterolemia: a review of the natural history, diagnosis, and management. *Cardiol Ther.* 2015;**4**:25–38.
62 Raal FJ, Honarpour N, Blom DJ, Hovingh GK, Xu F, Scott R, et al. Inhibition of PCSK9 with evolocumab in homozygous familial hypercholesterolaemia (TESLA Part B): a randomised, double-blind, placebo-controlled trial. *Lancet.* 2015;**385**:341–50.
63 Elbitar S, Susan-Resiga D, Ghaleb Y, El Khoury P, Peloso G, Stitziel N, et al. New sequencing technologies help revealing unexpected mutations in autosomal dominant hypercholesterolemia. *Sci Rep.* 2018;**8**:1943.
64 Andersen LH, Miserez AR, Ahmad Z, Andersen RL. Familial defective apolipoprotein B-100: a review. *J Clin Lipidol.* 2016;**10**:1297–302.
65 Hopkins PN, Defesche J, Fouchier SW, Bruckert E, Luc GäCR, Cariou B, et al. Characterization of autosomal dominant hypercholesterolemia caused by PCSK9 gain of function mutations and its specific treatment with Alirocumab, a PCSK9 monoclonal antibody. *Circ Cardiovasc Genet.* 2015;**8**:823–31.
66 Miserez AR, Keller U. Differences in the phenotypic characteristics of subjects with familial defective apolipoprotein B-100 and familial hypercholesterolemia. *Arterioscler Thromb Vasc Biol.* 1995;**15**:1719–29.
67 Shen H, Damcott CM, Rampersaud E, Pollin TI, Horenstein RB, Mcardle PF, et al. Familial defective apolipoprotein B-100 and increased low-density lipoprotein cholesterol and coronary artery calcification in the old order amish. *Arch Intern Med.* 2010;**170**:1850–5.
68 Bersot T, Russell S, Thatcher S, Pomernacki NK, Mahley RW, Weisgraber KH, et al. A unique haplotype of the apolipoprotein B-100 allele associated with familial defective apolipoprotein B-100 in a Chinese man discovered during a study of the prevalence of this disorder. *J Lipid Res.* 1993;**34**:1149–54.
69 Miserez AR, Muller PY. Familial defective apolipoprotein B-100: a mutation emerged in the Mesolithic ancestors of Celtic peoples? *Atherosclerosis.* 2000;**148**:433–6.
70 Peloso GM, Auer PL, Bis JC, Voorman A, Morrison AC, Stitziel NO, et al. Association of low-frequency and rare coding-sequence variants with blood lipids and coronary heart disease in 56,000 Whites and Blacks. *Am J Hum Genet.* 2014;**94**:223–32.
71 Benn M, Watts GF, Tybjærg-Hansen A, Nordestgaard BG. Mutations causative of familial hypercholesterolaemia: screening of 98 098 individuals from the Copenhagen General Population Study estimated a prevalence of 1 in 217. *Eur Heart J.* 2016;**37**:1384–94.
72 Tybjaerg-Hansen A, Steffensen R, Meinertz H, Schnohr P, Nordestgaard BG. Association of mutations in the apolipoprotein B gene with hypercholesterolemia and the risk of ischemic heart disease. *N Engl J Med.* 1998;**338**:1577–84.
73 Gallagher JJ, Myant NB. The affinity of low-density lipoproteins and of very-low-density lipoprotein remnants for the low-density lipoprotein receptor in homozygous

familial defective apolipoprotein B-100. *Atherosclerosis.* 1995;**115**:263–72.
74 März W, Ruzicka C, Pohl T, Usadel KH, Gross W. Familial defective apolipoprotein B-100: mild hypercholesterolaemia without atherosclerosis in a homozygous patient. *Lancet.* 1992;**340**:1362.
75 Loggen U, Boden A, Baron H, Schuster H, Tolle R, Netwich U, et al. Apolipoprotein B-100 gene mutations and cholesterol control in German patients. *Atherosclerosis.* 2003;**166**:411–2.
76 Ejarque I, Civer M, Francisco Ascaso J, Knecht E, Eugenia Armengod M, Carmen R, et al. [Identification and characterization of the first Spanish familial ligand-defective apolipoprotein B homozygote]. *Med Clin (Barc).* 2001;**116**:138–41.
77 Ceska R, Vrablík M, Horínek A. Familial defective apolipoprotein B-100: a lesson from homozygous and heterozygous patients. *Physiol Res.* 2000;**49**(Suppl 1):S125–30.
78 Hoogeveen RC, Gaubatz JW, Sun W, Dodge RC, Crosby JR, Jiang J, et al. Small dense low-density lipoprotein-cholesterol concentrations predict risk for coronary heart disease: the Atherosclerosis Risk In Communities (ARIC) study. *Arterioscler Thromb Vasc Biol.* 2014;**34**:1069–77.
79 Alves AC, Etxebarria A, Soutar AK, Martin C, Bourbon M. Novel functional APOB mutations outside LDL-binding region causing familial hypercholesterolaemia. *Hum Mol Genet.* 2014;**23**:1817–28.
80 Rabès JP, Varret M, Devillers M, Aegerter P, Villéger L, Krempf M, et al. R3531C mutation in the apolipoprotein B gene is not sufficient to cause hypercholesterolemia. *Arterioscler Thromb Vasc Biol.* 2000;**20**:E76–82.
81 Boren J, Lee I, Zhu W, Arnold K, Taylor S, Innerarity TL. Identification of the low density lipoprotein receptor-binding site in apolipoprotein B100 and the modulation of its binding activity by the carboxyl terminus in familial defective apo-B100. *J Clin Invest.* 1998;**101**:1084–93.
82 Borén J, Ekström U, Ågren B, Nilsson-Ehle P, Innerarity TL. The molecular mechanism for the genetic disorder familial defective apolipoprotein B100 *. *J Biol Chem.* 2001;**276**:9214–8.
83 Motazacker MM, Pirruccello J, Huijgen R, Do R, Gabriel S, Peter J, et al. Advances in genetics show the need for extending screening strategies for autosomal dominant hypercholesterolaemia. *Eur Heart J.* 2012;**33**:1360–6.
84 Bamshad MJ, Ng SB, Bigham AW, Tabor HK, Emond MJ, Nickerson DA, et al. Exome sequencing as a tool for Mendelian disease gene discovery. *Nat Rev Genet.* 2011;**12**:745–55.
85 Thomas ERA, Atanur SS, Norsworthy PJ, Encheva V, Snijders AP, Game L, et al. Identification and biochemical analysis of a novel APOB mutation that causes autosomal dominant hypercholesterolemia. *Mol Genet Genomic Med.* 2013;**1**:155–61.
86 Saint-Jore B, Varret M, Dachet C, Rabès JP, Devillers M, Erlich D, et al. Autosomal dominant type IIa hypercholesterolemia: evaluation of the respective contributions of LDLR and APOB gene defects as well as a third major group of defects. *Eur J Hum Genet.* 2000;**8**:621–30.
87 Varret M, Rabès JP, Saint-Jore B, Cenarro A, Marinoni JC, Civeira F, et al. A third major locus for autosomal dominant hypercholesterolemia maps to 1p34.1-p32. *Am J Hum Genet.* 1999;**64**:1378–87.
88 Hunt SC, Hopkins PN, Bulka K, Mcdermott MT, Thorne TL, Wardell BB, et al. Genetic localization to chromosome 1p32 of the third locus for familial hypercholesterolemia in a Utah kindred. *Arterioscler Thromb Vasc Biol.* 2000;**20**:1089–93.
89 Abi Fadel M, Villeger L, Rochaud S, Robin A, Rabès JP, Devillers M, et al. Fine mapping of region 1p32 that contains the third major locus for autosomal dominant hypercholesterolemia. *Am J Hum Genet.* 2001;**69**(suppl 4):539.
90 Boileau C, Abifadel M, Villeger L, Varret M, Rabès JP, Allard D, et al. An updated physical map encompassing the HCHOLA3 region at 1p34 and analysis of 37 functional and positional candidates. *Am J Hum Genet.* 2002;**408**(A1387):2002.
91 Seidah NG, Benjannet S, Wickham L, Marcinkiewicz J, Jasmin SB, Stifani S, et al. The secretory proprotein convertase neural apoptosis-regulated convertase 1 (NARC-1): liver regeneration and neuronal differentiation. *Proc Natl Acad Sci USA.* 2003;**100**:928–33.
92 Timms KM, Wagner S, Samuels ME, Forbey K, Goldfine H, Jammulapati S, et al. A mutation in PCSK9 causing autosomal-dominant hypercholesterolemia in a Utah pedigree. *Hum Genet.* 2004;**114**:349–53.
93 Haddad L, Day INM, Hunt S, Williams RR, Humphries SE, Hopkins PN. Evidence for a third genetic locus causing familial hypercholesterolemia: a non-LDLR, non-APOB kindred 1. *J Lipid Res.* 1999;**40**:1113–22.
94 Leren TP. Mutations in the PCSK9 gene in Norwegian subjects with autosomal dominant hypercholesterolemia. *Clin Genet.* 2004;**65**:419–22.
95 Sun X-M, Eden ER, Tosi I, Neuwirth CK, Wile D, Naoumova RP, et al. Evidence for effect of mutant PCSK9 on apolipoprotein B secretion as the cause of unusually severe dominant hypercholesterolaemia. *Hum Mol Genet.* 2005;**14**:1161–9.
96 Alghamdi RH, O'Reilly P, Lu C, Gomes J, Lagace TA, Basak A. LDL-R promoting activity of peptides derived from human PCSK9 catalytic domain (153–421): design, synthesis and biochemical evaluation. *Eur J Med Chem.* 2015;**92**:890–907.
97 Benjannet S, Rhainds D, Essalmani R, Mayne J, Wickham L, Jin W, et al. NARC-1/PCSK9 and its natural mutants: zymogen cleavage and effects on the low density lipoprotein (LDL) receptor and LDL cholesterol*. *J Biol Chem.* 2004;**279**:48865–75.
98 Essalmani R, Susan-Resiga D, Chamberland A, Abifadel M, Creemers JW, Boileau C, et al. In vivo evidence that furin from hepatocytes inactivates PCSK9. *J Biol Chem.* 2011;**286**:4257–63.
99 Lagace TA, Curtis DE, Garuti R, Mcnutt MC, Park SW, Prather HB, et al. Secreted PCSK9 decreases the number of LDL receptors in hepatocytes and in livers of parabiotic mice. *J Clin Invest.* 2006;**116**:2995–3005.
100 Ouguerram K, Chetiveaux M, Zair Y, Costet P, Abifadel M, Varret M, et al. Apolipoprotein B100 metabolism in autosomal-dominant hypercholesterolemia related to mutations in PCSK9. *Arterioscler Thromb Vasc Biol.* 2004;**24**:1448–53.
101 Abifadel M, Rabès J-P, Devillers M, Munnich A, Erlich D, Junien C, et al. Mutations and polymorphisms in the proprotein convertase subtilisin kexin 9 (PCSK9) gene in cholesterol metabolism and disease. *Hum Mutat.* 2009;**30**:520–9.
102 Abifadel M, Elbitar S, El Khoury P, Ghaleb Y, Chémaly M, Moussalli M-L, et al. Living the PCSK9 adventure: from the

identification of a new gene in familial hypercholesterolemia towards a potential new class of anticholesterol drugs. *Curr Atheroscler Rep.* 2014;**16**:439.
103 Abifadel M, Guerin M, Benjannet S, Rabès J-P, Le Goff W, Julia Z, et al. Identification and characterization of new gain-of-function mutations in the PCSK9 gene responsible for autosomal dominant hypercholesterolemia. *Atherosclerosis.* 2012;**223**:394–400.
104 Guo Q, Feng X, Zhou Y. PCSK9 variants in familial hypercholesterolemia: a comprehensive synopsis. *Front Genet.* 2020;**11**:1020.
105 Di Taranto MD, Benito-Vicente A, Giacobbe C, Uribe KB, Rubba P, Etxebarria A, et al. Identification and in vitro characterization of two new PCSK9 gain of function variants found in patients with familial hypercholesterolemia. *Sci Rep.* 2017;**7**:15282.
106 Abifadel M, Bernier L, Dubuc G, Nuel G, Rabes J-P, Bonneau J, et al. A PCSK9 variant and familial combined hyperlipidaemia. *J Med Genet.* 2008;**45**:780–6.
107 Cohen JC, Boerwinkle E, Mosley TH, Hobbs HH. Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. *New Engl J Med.* 2006;**354**:1264–72.
108 Cohen JC, Boerwinkle E, Mosley TH, Hobbs HH. Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. *N Engl J Med.* 2006;**354**:1264–72.
109 Humphries SE, Neely RDG, Whittall RA, Troutt JS, Konrad RJ, Scartezini M, et al. Healthy individuals carrying the PCSK9 p.R46L variant and familial hypercholesterolemia patients carrying PCSK9 p.D374Y exhibit lower plasma concentrations of PCSK9. *Clin Chem.* 2009;**55**:2153–61.
110 Berge KE, Ose L, Leren TP. Missense mutations in the PCSK9 gene are associated with hypocholesterolemia and possibly increased response to statin therapy. *Arterioscler Thromb Vasc Biol.* 2006;**26**:1094–100.
111 Karczewski KJ, Francioli LC, Tiao G, Cummings BB, Alföldi J, Wang Q, et al. The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature.* 2020;**581**(7809):434–43.
112 Leigh S. The PCSK9 gene homepage—Global Variome shared LOVD. https://databases.lovd.nl/shared/genes/PCSK9 (accessed July 4, 2022).
113 Gaudet D, Kereiakes DJ, McKenney JM, Roth EM, Hanotin C, Gipe D, et al. Effect of alirocumab, a monoclonal proprotein convertase subtilisin/kexin 9 antibody, on lipoprotein(a) concentrations (a pooled analysis of 150 mg every two weeks dosing from phase 2 trials). *Am J Cardiol.* 2014;**114**:711–5.
114 Raal FJ, Giugliano RP, Sabatine MS, Koren MJ, Langslet G, Bays H, et al. Reduction in lipoprotein(a) with PCSK9 monoclonal antibody evolocumab (AMG 145): a pooled analysis of more than 1,300 patients in 4 phase II trials. *J Am Coll Cardiol.* 2014;**63**:1278–88.
115 Blom DJ, Harada-Shiba M, Rubba P, Gaudet D, Kastelein JJP, Charng M-J, et al. Efficacy and safety of alirocumab in adults with homozygous familial hypercholesterolemia: the ODYSSEY HoFH trial. *J Am Coll Cardiol.* 2020;**76**:131–42.
116 Hovingh GK, Lepor NE, Kallend D, Stoekenbroek RM, Wijngaard PLJ, Raal FJ. Inclisiran durably lowers low-density lipoprotein cholesterol and proprotein convertase subtilisin/kexin type 9 expression in homozygous familial hypercholesterolemia. *Circulation.* 2020;**141**:1829–31.
117 Tibuakuu M, Blumenthal RS, Martin SS. American College of Cardiology. Bempedoic acid for LDL-C lowering: what do we know? https://www.acc.org/latest-in-cardiology/articles/2020/08/10/08/21/bempedoic-acid-for-ldl-c-lowering (accessed July 4 2022).
118 Kathiresan S, Melander O, Guiducci C, Surti A, Burtt N*LP, Rieder MJ, et al. Six new loci associated with blood low-density lipoprotein cholesterol, high-density lipoprotein cholesterol or triglycerides in humans. *Nat Genet.* 2008;**40**:189–97.
119 Raal FJ, Rosenson RS, Reeskamp LF, Hovingh GK, Kastelein JJP, Rubba P, et al. Evinacumab for homozygous familial hypercholesterolemia. *N Engl J Med.* 2020;**383**:711–20.
120 Marais AD. Familial hypercholesterolaemia. *Clin Biochem Rev.* 2004;**25**:49–68.
121 Narcisi TM, Shoulders CC, Chester SA, Read J, Brett DJ, Harrison GB, et al. Mutations of the microsomal triglyceride-transfer-protein gene in abetalipoproteinemia. *Am J Hum Genet.* 1995;**57**:1298–310.
122 Cuchel M, Meagher EA, du Toit Theron H, Blom DJ, Marais AD, Hegele RA, et al. Efficacy and safety of a microsomal triglyceride transfer protein inhibitor in patients with homozygous familial hypercholesterolaemia: a single-arm, open-label, phase 3 study. *Lancet.* 2013;**381**:40–6.
123 Raal FJ, Santos RD, Blom DJ, Marais AD, Charng M-J, Cromwell WC, et al. Mipomersen, an apolipoprotein B synthesis inhibitor, for lowering of LDL cholesterol concentrations in patients with homozygous familial hypercholesterolaemia: a randomised, double-blind, placebo-controlled trial. *Lancet.* 2010;**375**:998–1006.
124 D'Erasmo L, Gallo A, Cefalù AB, Di Costanzo A, Saheb S, Giammanco A, et al. Long-term efficacy of lipoprotein apheresis and lomitapide in the treatment of homozygous familial hypercholesterolemia (HoFH): a cross-national retrospective survey. *Orphanet J Rare Dis.* 2021;**16**:381.
125 Cesaro A, Fimiani F, Gragnano F, Moscarella E, Schiavo A, Vergara A, et al. New frontiers in the treatment of homozygous familial hypercholesterolemia. *Heart Fail Clin.* 2022;**18**:177–88.
126 Abifadel M, Pakradouni J, Collin M, Samson-Bouma M-E, Varret M, Rabès J-P, et al. Strategies for proprotein convertase subtilisin kexin 9 modulation: a perspective on recent patents. *Expert Opin Ther Pat.* 2010;**20**:1547–71.
127 Elbitar S, Khoury PE, Ghaleb Y, Rabès J-P, Varret M, Seidah NG, et al. Proprotein convertase subtilisin /kexin 9 (PCSK9) inhibitors and the future of dyslipidemia therapy: an updated patent review (2011–2015). *Expert Opin Ther Pat.* 2016;**26**:1377–92.
128 Mullard A. Merck readies oral, macrocyclic PCSK9 inhibitor for phase II test. *Nat Rev Drug Discov.* 2021;**21**:9.
129 Musunuru K, Chadwick AC, Mizoguchi T, Garcia SP, Denizio JE, Reiss CW, et al. In vivo CRISPR base editing of PCSK9 durably lowers cholesterol in primates. *Nature.* 2021;**593**:429–34.
130 Awan Z, Choi HY, Stitziel N, Ruel I, Bamimore MA, Husa R, et al. APOE p.Leu167del mutation in familial hypercholesterolemia. *Atherosclerosis.* 2013;**231**:218–22.
131 Wintjens R, Bozon D, Belabbas K, Mbou F, Girardet J-P, Tounian P, et al. Global molecular analysis and APOE mutations in a cohort of autosomal dominant hypercholesterolemia patients in France. *J Lipid Res.* 2016;**57**:482–91.

132 Wardell M, Rall S, Schaefer E, Kane J, Weisgraber K. Two apolipoprotein E5 variants illustrate the importance of the position of additional positive charge on receptor-binding activity. *J Lipid Res.* 1991;**32**:521–8.

133 Matsunaga A, Sasaki J, Moriyama K, Arakawa F, Takada Y, Nishi K, et al. Population frequency of apolipoprotein E5 (Glu3→Lys) and E7 (Glu244→Lys, Glu245→Lys) variants in western Japan. *Clin Genet.* 1995;**48**:93–9.

134 Yamamura T, Yamamoto A, Sumiyoshi T, Hiramori K, Nishioeda Y, Nambu S. New mutants of apolipoprotein E associated with atherosclerotic diseases but not to type III hyperlipoproteinemia. *J Clin Invest.* 1984;**74**:1229–37.

135 Tsuchiya S, Yamanouchi Y, Miyazaki R, Yanagi H, Yamakawa K, Yuzawa K, et al. Association of the apolipoprotein E4 allele with hypercholesterolemia in apparently healthy male adults in Tokyo. *Jap J Human Genet.* 1987;**32**:283–9.

136 Ruzicka V, März W, Russ A, Fisher E, Mondorf W, Gross W. Characterization of the gene for apolipoprotein E5-Frankfurt (Gln81→Lys, Cys112→Arg) by polymerase chain reaction, restriction isotyping, and temperature gradient gel electrophoresis. *Electrophoresis.* 1993;**14**:1032–7.

137 Kitahara M, Shinomiya M, Shirai K, Saito Y, Yoshida S. Frequency and role of apo E phenotype in familial hypercholesterolemia and non-familial hyperlipidemia in the Japanese. *Atherosclerosis.* 1990;**82**:197–204.

138 Yanagi K, Yamashita S, Hiraoka H, Ishigami M, Kihara S, Hirano K-I, et al. Increased serum remnant lipoproteins in patients with apolipoprotein E7 (apo E Suita). *Atherosclerosis.* 1997;**131**:49–58.

139 van den Maagdenberg AM, Weng W, de Bruijn IH, de Knijff P, Funke H, Smelt AHM, et al. Characterization of five new mutants in the carboxyl-terminal domain of human apolipoprotein E: no cosegregation with severe hyperlipidemia. *Am J Hum Genet.* 1993;**52**:937–46.

140 Faivre L, Saugier-Veber P, Pais de Barros J-P, Verges B, Couret B, Lorcerie B, et al. Variable expressivity of the clinical and biochemical phenotype associated with the apolipoprotein E p.Leu149del mutation. *Eur J Hum Genet.* 2005;**13**:1186–91.

141 Fouchier SW, Dallinga-Thie GM, Meijers JCM, Zelcer N, Kastelein JJP, Defesche JC, et al. Mutations in STAP1 are associated with autosomal dominant hypercholesterolemia. *Circ Res.* 2014;**115**:552–5.

142 Danyel M, Ott C-E, Grenkowitz T, Salewsky B, Hicks AA, Fuchsberger C, et al. Evaluation of the role of STAP1 in familial hypercholesterolemia. *Sci Rep.* 2019;**9**:11995.

143 Loaiza N, Hartgers ML, Reeskamp LF, Balder J-W, Rimbert A, Bazioti V, et al. Taking one step back in familial hypercholesterolemia: STAP1 does not alter plasma LDL (low-density lipoprotein) cholesterol in mice and Humans. *Arterioscler Thromb Vasc Biol.* 2020;**40**:973–85.

144 Hegele RA, Knowles JW, Horton JD. Delisting STAP1: the rise and fall of a putative hypercholesterolemia gene. *Arterioscler Thromb Vasc Biol.* 2020;**40**:847–9.

145 Marques-Pinheiro A, Marduel M, Rabès J-P, Devillers M, Villéger L, Allard D, et al. A fourth locus for autosomal dominant hypercholesterolemia maps at 16q22.1. *Eur J Hum Genet.* 2010;**18**:1236–42.

146 Cenarro A, García-Otín A-L, Tejedor MT, Solanas M, Jarauta E, Junquera C, et al. A presumptive new locus for autosomal dominant hypercholesterolemia mapping to 8q24.22. *Clin Genet.* 2011;**79**:475–81.

147 Stitziel NO, Peloso GM, Abifadel M, Cefalu AB, Fouchier S, Motazacker MM, et al. Exome sequencing in suspected monogenic dyslipidemias. *Circ Cardiovasc Genet.* 2015;**8**:343–50.

148 Khachadurian AK, Uthman SM. Experiences with the homozygous cases of familial hypercholesterolemia. A report of 52 patients. *Nutr Metab.* 1973;**15**:132–40.

149 Soutar A, Naoumova R, Traub L. Genetics, clinical phenotype, and molecular cell biology of autosomal recessive hypercholesterolemia. *Arterioscler Thromb Vasc Biol.* 2003;**23**:1963–70.

150 Mishra SK, Watkins SC, Traub LM. The autosomal recessive hypercholesterolemia (ARH) protein interfaces directly with the clathrin-coat machinery. *Proc Natl Acad Sci USA.* 2002;**99**:16099–104.

151 He G, Gupta S, Yi M, Michaely P, Hobbs HH, Cohen JC. ARH is a modular adaptor protein that interacts with the LDL receptor, clathrin, and AP-2. *J Biol Chem.* 2002;**277**:44044–9.

152 Leigh S. The LDLRAP1 gene homepage—Global Variome shared LOVD. https://databases.lovd.nl/shared/genes/LDLRAP1 (accessed July 4, 2022).

153 Fellin R, Arca M, Zuliani G, Calandra S, Bertolini S. The history of autosomal recessive hypercholesterolemia (ARH). From clinical observations to gene identification. *Gene.* 2015;**555**:23–32.

154 Filigheddu F, Quagliarini F, Campagna F, Secci T, Degortes S, Zaninello R, et al. Prevalence and clinical features of heterozygous carriers of autosomal recessive hypercholesterolemia in Sardinia. *Atherosclerosis.* 2009;**207**:162–7.

155 Pisciotta L, Priore Oliva C, Pes GM, Di Scala L, Bellocchio A, Fresa R, et al. Autosomal recessive hypercholesterolemia (ARH) and homozygous familial hypercholesterolemia (FH): a phenotypic comparison. *Atherosclerosis.* 2006;**188**:398–405.

156 Naoumova RP, Neuwirth C, Lee P, Miller JP, Taylor KG, Soutar AK. Autosomal recessive hypercholesterolaemia: long-term follow up and response to treatment. *Atherosclerosis.* 2004;**174**:165–72.

157 Sjouke B, Kusters DM, Kindt I, Besseling J, Defesche JC, Sijbrands EJG, et al. Homozygous autosomal dominant hypercholesterolaemia in the Netherlands: prevalence, genotype-phenotype relationship, and clinical outcome. *Eur Heart J.* 2015;**36**:560–5.

158 Baum SJ, Sijbrands EJG, Mata P, Watts GF. The doctor's dilemma: challenges in the diagnosis and care of homozygous familial hypercholesterolemia. *J Clin Lipidol.* 2014;**8**:542–9.

159 Foody JM, Vishwanath R. Familial hypercholesterolemia/autosomal dominant hypercholesterolemia: molecular defects, the LDL-C continuum, and gradients of phenotypic severity. *J Clin Lipidol.* 2016;**10**:970–86.

160 Kamar A, Khalil A, Nemer G. The digenic causality in familial hypercholesterolemia: revising the genotype-phenotype correlations of the disease. *Front Genet.* 2020;**11**:572045.

161 Allard D, Amsellem S, Abifadel M, Trillard M, Devillers M, Luc G, et al. Novel mutations of the PCSK9 gene cause variable phenotype of autosomal dominant hypercholesterolemia. *Hum Mutat.* 2005;**26**:497.

162 Pisciotta L, Priore Oliva C, Cefalù AB, Noto D, Bellocchio A, Fresa R, et al. Additive effect of mutations in LDLR and PCSK9 genes on the phenotype of familial hypercholesterolemia. *Atherosclerosis.* 2006;**186**:433–40.

163 Tada H, Kawashiri M-A, Nohara A, Inazu A, Kobayashi J, Mabuchi H, et al. Autosomal recessive hypercholesterolemia: a mild phenotype of familial hypercholesterolemia: insight from the kinetic study using stable isotope and animal studies. *J Atheroscler Thromb.* 2015;**22**:1–9.

164 Cameron J, Holla ØL, Ranheim T, Kulseth MA, Berge KE, Leren TP. Effect of mutations in the PCSK9 gene on the cell surface LDL receptors. *Hum Mol Genet.* 2006;**15**:1551–8.

165 Horton JD, Cohen JC, Hobbs HH. PCSK9: a convertase that coordinates LDL catabolism. *J Lipid Res.* 2009;**50**(Suppl S1):72–177.

166 Mabuchi H, Nohara A, Noguchi T, Kobayashi J, Kawashiri M-A, Inoue T, et al. Genotypic and phenotypic features in homozygous familial hypercholesterolemia caused by proprotein convertase subtilisin/kexin type 9 (PCSK9) gain-of-function mutation. *Atherosclerosis.* 2014;**236**:54–61.

167 Mabuchi H, Nohara A, Noguchi T, Kobayashi J, Kawashiri M-A, Tada H, et al. Molecular genetic epidemiology of homozygous familial hypercholesterolemia in the Hokuriku district of Japan. *Atherosclerosis.* 2011;**214**:404–7.

168 Bayona A, Arrieta F, Rodríguez-Jiménez C, Cerrato F, Rodríguez-Nóvoa S, Fernández-Lucas M, et al. Loss-of-function mutation of PCSK9 as a protective factor in the clinical expression of familial hypercholesterolemia: a case report. *Medicine (Baltimore).* 2020;**99**:e21754.

169 Musunuru K, Pirruccello JP, Do R, Peloso GM, Guiducci C, Sougnez C, et al. Exome sequencing, ANGPTL3 mutations, and familial combined hypolipidemia. *N Engl J Med.* 2010;**363**:2220–7.

170 Futema M, Plagnol V, Li K, Whittall RA, Neil HAW, Seed M, et al. Whole exome sequencing of familial hypercholesterolaemia patients negative for LDLR/APOB/PCSK9 mutations. *J Med Genet.* 2014;**51**:537–44.

171 Cohen JC. Emerging LDL therapies: using human genetics to discover new therapeutic targets for plasma lipids. *J Clin Lipidol.* 2013;**7**:S1–5.

172 Willer CJ, Schmidt EM, Sengupta S, Bierhals AJ, Vourakis AC, Sperry AE, et al. Discovery and refinement of loci associated with lipid levels. *Nat Genet.* 2013;**45**:1274–83.

173 Stitziel NO, Khera AV, Wang X, Bierhals AJ, Vourakis AC, Sperry AE, et al. ANGPTL3 deficiency and protection against coronary artery disease. *J Am Coll Cardiol.* 2017;**69**:2054–63.

174 Chilazi M, Sharma G, Blumenthal RS, Martin SS. American College of Cardiology. Angiopoietin-like 3 (ANGPTL3)—a novel therapeutic target for treatment of hyperlipidemia. https://www.acc.org/latest-in-cardiology/articles/2021/01/06/13/01/angiopoietin-like-3-angptl3 (accessed July 4, 2022).

175 Cupido AJ, Tromp TR, Hovingh GK. The clinical applicability of polygenic risk scores for LDL-cholesterol: considerations, current evidence and future perspectives. *Curr Opin Lipidol.* 2021;**32**:112–6.

176 Klarin D, Damrauer SM, Cho K, Sun YV, Teslovich TM, Honerlaw J, et al. Genetics of blood lipids among ~300,000 multi-ethnic participants of the Million Veteran Program. *Nat Genet.* 2018;**50**:1514–23.

177 Graham SE, Clarke SL, Wu K-HH, Kanoni S, Zajac GJM, Ramdas S, et al. The power of genetic diversity in genome-wide association studies of lipids. *Nature.* 2021;**600**:675–9.

178 Taylor A, Wang D, Patel K, Whittall R, Wood G, Farrer M, et al. Mutation detection rate and spectrum in familial hypercholesterolaemia patients in the UK pilot cascade project. *Clin Genet.* 2010;**77**:572–80.

179 Humphries SE, Whittall RA, Hubbart CS, Maplebeck S, Cooper JA, Soutar AK, et al. Genetic causes of familial hypercholesterolaemia in patients in the UK: relation to plasma lipid levels and coronary heart disease risk. *J Med Genet.* 2006;**43**:943–9.

180 Graham CA, McIlhatton BP, Kirk CW, Beattie ED, Lyttle K, Hart P, et al. Genetic screening protocol for familial hypercholesterolemia which includes splicing defects gives an improved mutation detection rate. *Atherosclerosis.* 2005;**182**:331–40.

181 Talmud PJ, Shah S, Whittall R, Futema M, Howard P, Cooper JA, et al. Use of low-density lipoprotein cholesterol gene score to distinguish patients with polygenic and monogenic familial hypercholesterolaemia: a case-control study. *Lancet.* 2013;**381**:1293–301.

182 Teslovich TM, Musunuru K, Smith AV, Edmondson AC, Stylianou IM, Koseki M, et al. Biological, clinical and population relevance of 95 loci for blood lipids. *Nature.* 2010;**466**:707–13.

183 Futema M, Shah S, Cooper JA, Li K, Whittall RA, Sharifi M, et al. Refinement of variant selection for the LDL cholesterol genetic risk score in the diagnosis of the polygenic form of clinical familial hypercholesterolemia and replication in samples from 6 countries. *Clin Chem.* 2015;**61**:231–8.

184 Ghaleb Y, Elbitar S, El Khoury P, Bruckert E, Carreau V, Carrié A, et al. Usefulness of the genetic risk score to identify phenocopies in families with familial hypercholesterolemia? *Eur J Hum Genet.* 2018;**26**:570–8.

185 Trinder M, Francis GA, Brunham LR. Association of monogenic vs polygenic hypercholesterolemia with risk of atherosclerotic cardiovascular disease. *JAMA Cardiol.* 2020;**5**:390–9.

186 Sjouke B, Tanck MWT, Fouchier SW, Defesche JC, Hutten BA, Wiegman A, et al. Children with hypercholesterolemia of unknown cause: value of genetic risk scores. *J Clin Lipidol.* 2016;**10**:851–9.

187 Paquette M, Chong M, Thériault S, Dufour R, Paré G, Baass A. Polygenic risk score predicts prevalence of cardiovascular disease in patients with familial hypercholesterolemia. *J Clin Lipidol.* 2017;**11**:725–32.e5.

188 Paquette M, Baass A. Polygenic risk scores for cardiovascular disease prediction in the clinical practice: are we there? *Atherosclerosis.* 2022;**340**:46–7.

189 Trinder M, Li X, DeCastro ML, Cermakova L, Sadananda S, Jackson LM, et al. Risk of premature atherosclerotic disease in patients with monogenic versus polygenic familial hypercholesterolemia. *J Am Coll Cardiol.* 2019;**74**:512–22.

190 Trinder M, Paquette M, Cermakova L, Ban MR, Hegele RA, Baass A, et al. Polygenic contribution to low-density lipoprotein cholesterol levels and cardiovascular risk in monogenic familial hypercholesterolemia. *Circ Genom Precis Med.* 2020;**13**:515–23.

191 Sharifi M, Futema M, Nair D, Humphries SE. Polygenic hypercholesterolemia and cardiovascular disease risk. *Curr Cardiol Rep.* 2019;**21**:43.
192 Sharifi M, Higginson E, Bos S, Gallivan A, Harvey D, Li KW, et al. Greater preclinical atherosclerosis in treated monogenic familial hypercholesterolemia vs. polygenic hypercholesterolemia. *Atherosclerosis.* 2017;**263**:405–11.
193 Watts GF, Gidding S, Wierzbicki AS, Toth PP, Alonso R, Brown WV, et al. Integrated guidance on the care of familial hypercholesterolaemia from the International FH Foundation. *Int J Cardiol.* 2014;**171**:309–25.
194 Mach F, Baigent C, Catapano AL, Koskinas KC, Casula M, Badimon L, et al. 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. *Eur Heart J.* 2020;**41**:111–88.
195 Visseren FLJ, Mach F, Smulders YM, Carballo D, Koskinas KC, Bäck M, et al. 2021 ESC Guidelines on cardiovascular disease prevention in clinical practice. *Eur Heart J.* 2021;**42**:3227–337.
196 Girardet J-P, Luc G, Rieu D, Bruckert E, Darmaun D, Farnier M. Prise en charge des hypercholestérolémies de l'enfant: recommandations du Comité de nutrition de la Société française de pédiatrie et de la Nouvelle société française d'athérosclérose. *Arch Pédiatr.* 2011;**18**:217–29.
197 Sturm AC, Knowles JW, Gidding SS, Ahmad ZS, Ahmed CD, Ballantyne CM, et al. Clinical genetic testing for familial hypercholesterolemia: JACC Scientific Expert Panel. *J Am Coll Cardiol.* 2018;**72**:662–80.
198 Chora JR, Iacocca MA, Tichý L, Wand H, Kurtz CL, Zimmermann H, et al. The Clinical Genome Resource (ClinGen) Familial Hypercholesterolemia Variant Curation Expert Panel consensus guidelines for LDLR variant classification. *Genet Med.* 2022;**24**:293–306.
199 Brown EE, Sturm AC, Cuchel M, Braun LT, Duell PB, Underberg JA, et al. Genetic testing in dyslipidemia: a scientific statement from the National Lipid Association. *J Clin Lipidol.* 2020;**14**:398–413.
200 Correia M, Kagenaar E, van Schalkwijk DB, Bourbon M, Gama-Carvalho M. Machine learning modelling of blood lipid biomarkers in familial hypercholesterolaemia versus polygenic/environmental dyslipidaemia. *Sci Rep.* 2021;**11**:3801.
201 Pina A, Helgadottir S, Mancina RM, Pavanello C, Pirazzi C, Montalcini T, et al. Virtual genetic diagnosis for familial hypercholesterolemia powered by machine learning. *Eur J Prev Cardiol.* 2020;**27**:1639–46.
202 Miller DM, Gaviglio A, Zierhut HA. Development of an implementation framework for overcoming underdiagnoses of familial hypercholesterolemia in the USA. *Public Health Genomics.* 2021;**24**:110–22.
203 Ibrahim S, Reeskamp LF, Stroes ESG, Watts GF. Advances, gaps and opportunities in the detection of familial hypercholesterolemia: overview of current and future screening and detection methods. *Curr Opin Lipidol.* 2020;**31**:347–55.

Correspondence: Marianne Abifadel and Catherine Boileau, UMR1148, Inserm, Hôpital Bichat-Claude Bernard, 46 rue Henri Huchard, F-75018 Paris, France.
Email: catherine.boileau@inserm.fr

