REVIEW ARTICLE

Familial Hypercholesterolemia: From Diagnosis to Treatment

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Abstract

Familial hypercholesterolemia (FH) is an inherited common autosomal Mendelian disorder of lipoprotein metabolism with a population prevalence of 1 in 500. FH is characterized by severely elevated levels of low-density lipoprotein cholesterol (LDL-C), which result in surplus deposition of cholesterol in tissues. This condition leads to premature at hero sclerosis and early-onset of coronary heart disease. FH mainly results from mutations in the *LDLR* gene. However, mutations in other genes like *APOB* and *PCSK9* can cause similar phenotype. Early diagnosis and treatment of FH patients will reduce morbidity and mortality. Index cases are usually diagnosed using cholesterol levels, clinical characteristics and familial history; despite this, genetic testing may present a decisive diagnosis of FH by detecting a pathological mutation. Then cascade testing is implemented for first-degree relatives by using lipid levels and genetic tests. This is the most cost-effective strategy performed in some countries. Statins are the first-line treatment in most of the patients for LDL-C reduction. Nonetheless, many FH patients cannot attain to the normal LDL-C levels with statins consumption. For these patients several new classes of pharmacotherapy and novel strategies exist to obtain greater LDL-C reductions such as ezetimibe, colesevelam, thyroid hormone analogs, microsomal triglyceride transfer protein inhibitors, apolipoprotein B100 antisense and *PCSK9*-specific monoclonal antibodies. In this article we review familial hypercholesterolemia, its diagnostic methods and genetic tests, new pharmacotherapies and novel strategies in the management of familial hypercholesterolemia.

Keywords: Diagnosis, Familial hypercholesterolemia, LDL receptor, Treatment

Introduction

Familial hypercholesterolemia (FH (OMIM) 143890) is the most common genetic disorder of low density lipoprotein (LDL) metabolism associated with premature cardiovascular complications (1,2). As a result, in FH patients, life expectancy is reduced approximately 20 to 30 years because of sudden death and myocardial infarction. Unfortunately, the majority of FH cases are undiagnosed or just diagnosed after their first coronary event (3,4). In 1939, Dr Carl Muller, a Norwegian physician discovered

characteristics of FH and its correlation with atherosclerosis (5). In 1964, Khachadurian, for the first time, showed that the inheritance pattern of FH is autosomal co-dominant with a genedos age effect. He segregated patients from affected families into three groups: supposed homozygotes with plasma cholesterol concentrations four times higher than normal; heterozygotes with plasma cholesterol levels two times higher than normal; and normal individuals (6).

Epidemiology:

Worldwide prevalence of FH is estimated about 1 in 500 for heterozygotes (0.2%), 1 in 200,000 for compound heterozygotes and 1 in 1 million for homozygous (7,8). In certain communities, such as French Canadians, Finns, Afrikaners, Druze and Lebanese, FH frequency can be as high as 1 per 67 in the heterozygous form, and 1 per 10,000 in the homozygous form because of founder effects (9-16). It appears that there are over 13 million people worldwide and ~620,000 in the United States who have FH (2,7,8). Most of our knowledge is based on studies done in the west. There are only few genetic studies reported from Asia and also the spectrum of mutations is different from the European spectrum. Unfortunately there are no true estimates of identified FH cases in Asia. Patients coming in to hospitals are being screened for their lipid profile (HDL/LDL/cholesterol/..) and if they are at high risk levels, routine treatments will be done for the patients and nobody suspect that it could be FH or not. So Asian communities need to identify the population frequency of prevalent haplotypes and adopt the appropriate genetic tests by government in the national level for screening and management of FH cases (8,9).

Biochemical and clinical characteristics:

Familial hypercholesterolemia causes the elevation of total serum cholesterol and serum LDL-C to the high risk levels. Plasma levels of important lipoprotein particles such as LDL-C levels are the main determinants of the initiation of changes in vascular endothelial damage leading to atherosclerotic lesions, peripheral arterial disease, premature coronary artery disease (CAD), and aortic stenosis.

There is no single internationally accepted set of criteria for the clinical diagnosis of FH but 3 common sets of criteria are used in lipid clinics and researches in the world (Details are given in table 1):

1. Simon Broome criteria (UK)
2. MEDPED (Make Early Diagnosis to Prevent Early Death) (USA)
3. Dutch Lipid Clinic criteria (Netherland)

Also, FH leads to accumulation of cholesterol in the skin, which known as Xanthomas. Xanthomas exclusively affect the tendons: elbows, Achilles tendon and hands. Xanthelasmata are another clinical signs of FH. Indeed, they are lipid depositions on the Eyelids. Sediment of lipid can also occur in the corneas that causepresenile corneal arcus (Figure 1). Xanthomas and corneal arcus are path gnomonic for heterozygous FH (HeFH)

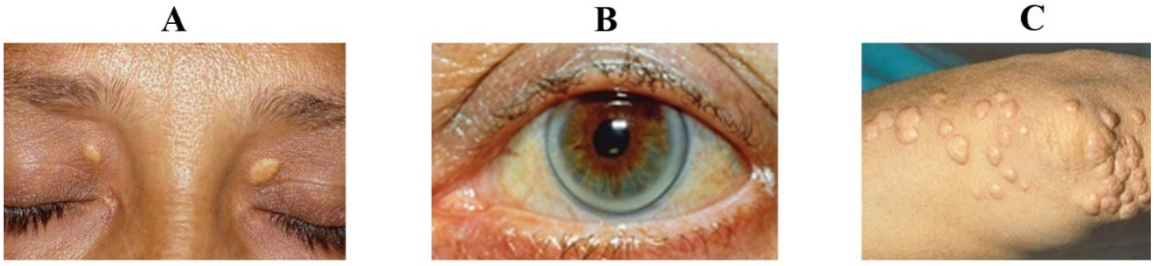

Figure1. clinical manifestations of familial hypercholesterolemia. (**A**) Xanthelasma (yellowish deposit of fat usually on or around the eyelids). (**B**) Corneal arcus (white, grey, or blue ring in front of the periphery of the iris). (**C**) Xanthomas(deposit of fat found in the tendons of the hands, feet, and heel) (19,20).

Table 1. Criteria for The Clinic al Diagnosis of Familial Hypercholesterolemia

UK: Simon Broome criteria			
Total-cholesterol (LDL-C) in mg/dl >260(155) in patients with age <18 years and>290 (190) in patients >18 years	AND	Family history of elevated total-cholesterol >290 mg/dl in first or second degree relative OR Family history of coronary disease at age <60 years in first degree relative or <50 years in second degree relative	Possible FH
		Tendon xanthomas in the patient or in first or second degree relative	Probable FH
		DNA mutation consistent with FH	Definite FH

USA:MEDPED criteria (87% sensitivity and 98% specificity)

Age in years	**Total-cholesterol (LDL-C) in mg/dl**			
	General population	**First degree relative**	**Second degree relative**	**Third degree relative**
<18	270 (200)	220 (155)	230 (165)	240 (170)
18-29	290 (220)	240 (170)	250 (185)	260 (185)
30-39	340 (240)	270 (190)	280 (200)	290 (210)
≥40	360 (260)	290 (205)	300 (215)	310 (225)

Netherland: Dutch Lipid Clinic criteria

LDLR gene functional mutation or LDL-cholesterol >330 mg/dl	8 points	Definite FH ≥8 points
Presence of tendonxanthoma	6 points	
LDL-C between 250 and 329 mg/dl	5 points	Probable FH 6-7 points
Presence of arcus cornea eatage<45years	4 points	
LDL-C between 190 and 249 mg/dl	3 points	
Personal history of CAD or First degree relative age <18years with LDL-C >95th percentile or First degree relative with tendon xanthoma or arcus corneae	2 points	Possible FH 3-5points
LDL-C between 190 and 249 mg/dl or Personal history of premature cerebral or peripheral artery disease or First degree adult relative with premature CAD or DL-C>95th percentile	1 points	

LDL-C: Low-density lipoprotein-cholesterol, FH: Familial hypercholesterolemia, DNA: Deoxyribonucleic acid, CAD: Coronary artery disease, DLR: LDL receptor

and homozygous FH (hoFH) but in hoFH patients are much more severe and occur at early ages (ex: observed at birth or during early childhood). The presence of these symptoms is correlated with a three-fold higher risk of cardiovascular disease (CVD). It should be considered that most of the HeFH cases have not Xanthomas, Xanthelasmata or corneal arcus especially in young ages (17,18).

Diagnosis and case finding:

It should be noticed that some diseases have the same symptom with FH; so, correct diagnosis especially for HeFH is so important. The diagnosis of a HeFH case is based on identification of high LDL levels and evidence of an autosomal dominant pattern of inheritance. Before the diagnosis, secondary causes of hypercholesterolemia such as hypothyroidism and proteinuria should be ruled out. Familial combined hyperlipoproteinemia is more rampant than HeFH with the prevalence of 1 in 100 to 1 in 50. But, the pattern of inheritance is not as bright as HeFH. HeFH is different in several aspects:

1. It is less common than familial combined hyperlipidemia or polygenic hyperlipidemia.
2. It has an autosomal dominant mode of inheritance with over 90% penetrance.
3. Children are affected and need to be treated.
4. The therapeutic strategies are different from more common forms of hypercholesterolemia. For example, obesity is common in familial combined and polygenic hypercholesterolemia, and weight loss may reduce LDL level. However, obesity in HeFH is approximately uncommon, and weight reduction is less effective to treatment.
5. Population-based screening for HeFH is relatively inefficient. Studies have shown at total cholesterol of 310 mg/dL, just 4% of the general population will have HeFH, whereas among first degree relatives of a person with HeFH, 95% of persons with a level of 310 mg/dL will be HeFH. Hence, family-based screening has more acceptable results. During the selection of patients some other parameters such as age, gender, high density lipoprotein, lipoprotein (a), blood pressure status, smoking

Table 2. Heterozygous Familial Hypercholesterolemia (HeFH) and Familial Combined Hyperlipidemia (FCHL)

Similarities	**HeFH**	**FCHL**
High LDL cholesterol and early CHD	+	+
Family aggregation	+	+
Men tend to have CHD events l0-20 years earlier than women	+	+
Have multiplicative interactions with smoking and other risk factors	+	+
Respond to HMG Co-A reductase inhibitors	+	+
Normal lipid levels can often result from drug and diet therapy	+	+
Treatment prevents progression of coronary atherosclerosis	+	+
Differences	**HeFH**	**FCHL**
Clear autosomal dominant mode of inheritance	+	-
Very high total and LDL-cholesterol in early childhood	+	-
Gene testing currently available can correctly classify borderline cases	+	-
Have many affected close and distant relatives	+	-
High triglyceride and low HDL cholesterol	+	-
Often associated with hypertension, hyper insulinemia and diabetes	-	+
Quite responsive to exercise in many persons	-	+
Associated with obesity and responsive to weight loss	-	+
Often requires 3 medications to normalize LDL cholesterol	+	-
Both men and women with this disorder can die because of coronary disease in late 20's	+	-
Approximate frequency in the general population	1:500	1:100
Average age of myocardial infarction in untreated men	45	55

status, thyroid hormones and diabetes should be considered (21-23).

Molecular pathways that develop familial hypercholesterolaemia:

In 1985 Michael Brown and Joseph Goldstein won the Nobel Prize for revealing mechanism of the regulation of cholesterol metabolism in human (24). The LDL receptor is a cell-surface glycoprotein that mediates the uptake of plasma LDL. It is synthesized as an immature protein in Endoplasmic reticulum then is processed in the Golgi apparatus for maturation and after that it is transported to the cell surface. There, the receptor specifically binds apolipoprotein B that carries LDL particles in the extracellular fluid. Then the receptor–ligand complex is entered to the cell by endocytosis via clathrin-coated pits through interactions involving the LDL receptor adaptor protein (*LDLRAP1*). The complex is transferred via early endosomes to the late endosomal compartment, where the receptor–ligand complex is segregated in its acidic environment. The receptor is recycled to the cell surface while the LDL particle is decomposed in the lysosomal compartment. Reposition of free cholesterol released by hydrolysis of cholesteryl esters inactivates a transcription factor that is called sterol regulatory element binding protein (*SREBP*). *SREBP* stimulates expression of genes involved in cholesterol synthesis and the LDL receptor. So the LDL-receptor pathway preserves intracellular cholesterol homeostasis. The proprotein convertase subtilisin/kexin 9 *(PCSK9*), lessens the LDL-protein content of cells by a post-translational mechanism that is not well understood (25,26).

Causative mutations in familial hypercholesterolemia:

➢ ***Low-density lipoprotein receptor gene(LDLR)(OMIM 606945):***

In1970s Brown and Goldstein found that the FH phenotype caused by defective endocytosis

Table 3. Total Cholesterol and (LDL) Criteria for Diagnosing Probable HFH

Age	**Total Cholesterol (LDL)**			
	First Relative	**Second Relative**	**Third Relative**	**General Population**
<18	220 (155)	230 (165)	240(170)	270 (200)
20	240 (170)	250 (180)	260 (185)	290(220)
30	270 (190)	280 (200)	290(210)	340 (240)
≥ 40	290 (205)	300 (215)	310(225)	360(260)

of low-density lipoprotein (LDL) cholesterol by its receptor (*LDLR*) that results from mutations in the *LDLR* gene (26-28). This gene is about45 kilobases, has 18 exons, and maps to the short arm of chromosome 19 (19p13.1-p13.3) and encode an 860-amino acid LDL receptor protein (29, 30). Mutations in this gene known as first and main cause of FH. Mutations involving a small number of nucleotides, from point mutations to small deletions, insertions or intronic mutations account for 90% of all mutations in the *LDLR* gene. From July 1, 2003 till now over 1700 *LDLR* variants have been identified in FH cases. It should be noticed that all of these variants are not functional mutations. All this gene variants are compiled online at two websites: http://www.ucl.ac.uk/fh/ and http://www.umd.be/LDLR/. Among these small DNA variations of the *LDLR* gene, 58.5% are missense, 21.7% are small deletions or insertions, 10.4 % are nonsense and 9.4% are splice site mutations (31,32).

- ***ApoB-100 gene (APOB100) (OMIM 107730):***

In the late 1980s Grundy, Innerarity and their colleagues found that the same clinical phenotype could also be due to mutations in the *APOB* gene that has been called "familial defective apolipoprotein B-100"(FDB, OMIM 144010) (33,34). *APOB* gene spans 43 kilobases, has 29 exons and is located on chromosome2 (2p23-24) (35-37). The produced 4,536-amino acid protein is the only protein component of LDL particles and serves as the ligand for the LDL receptor protein(38). Most cases with familial ligand-defective ApoB in Europe are heterozygous for a point mutation that cause a single amino acid substitution of Arginine 3500 with glutamine (Arg3500Gln) in its LDL-receptor-binding domain (33). A rare substitution in the same cod on as the first mutation resulting in substitution of Arginine with tryptophan (Arg3500Trp) (39). This mutation is rare in Europe, but is relatively common in the Chinese population (40).

- ***Pro-protein convertase subtilisin/ kexin 9 gene(PCSK9) (OMIM 607786):***

In 2003 Abifadel and their colleagues found this type of mutation in a French family.*PCSK9* gene are located on chromosome 1p32 (41). Mutations in *PCSK9* have been demonstrated tocosegregate with severe hypercholesterolemia in a number of families in different countries but remain a rare cause of FH (16,42-45).

- ***LDLR adaptor protein 1 gene (LDLRAP1) (OMIM 605747):***

In 1964, Khachadurian pointed out that the pattern of inheritance in a small number of families with severe hypercholesterolemia seems to be recessive. This disorder was called autosomal recessive hypercholesterolemia (ARH, OMIM 603813) (6). In this patient there is no detectable *LDLR* defect. At the beginning of the 21st century, researchers found that recessive null mutations in a novel gene called LDL receptor adaptor protein 1 (*LDLRAP1*) can cause this form of hypercholesterolemia (46,47). The phenotype of ARH is similar but milder than homozygous FH and shows better reply to treatment with lipid-lowering medications. The phenotype also tends to be variable in patients from the same family (48).

- ***Another genes:***

Despite all the developments in mutation detection technology, when some definite FH cases are screened for common mutations, no results will be achieved. In these cases, mutations in other candidate genes are recognized that are very rare such as: *CYP7A1*, which encodes the enzyme that catalyzes the first step in the hepatic catabolism of cholesterol (49), sterol regulatory element binding protein (*SREBP*) and the *SREBP* cleavage-activating protein (*SCAP*) isoforms (50-52).

Types of technique for genetic screening of FH:

Generally, genetic testing for *LDLR*, *APO B*and recently for *PCSK9* gene mutations is the preferable diagnostic strategy. It should be

considered that mutations in affected individuals are different based on their race and geographical location, except when a founder effect is present in the population. The gold-standard approach for genetic analysis of patients is full nucleotide sequencing of the causative genes or combination of multiplex ligation-dependent probe amplification (MLPA) together with either exon-by-exon sequencing. Other basic approaches include the use of a selected panel of common mutations or gene screening for known and unknown mutations by using denaturing high performance liquid chromatography (D-HPLC) or high resolution melting (HRM) (53-55). These methods often are applied for *LDLR* gene. Older molecular methods used for detection of *LDLR* mutations are single-strand conformation polymorphism (SSCP) analysis (56-59), denaturing gradient gel electrophoresis (DGGE) (60,61), and denaturing HPLC (DHPLC) (62). Recent approaches include sequencing of the *LDLR* cDNA region (63), a SSCP/heteroduplex method followed by capillary electrophoresis (64) and melt-micro plate array diagonal gel electrophoresis, which are appropriate for the detection of novel mutations (65). Finally, exome sequencing can increase our understanding about the etiology of this complex heterogeneous disorder specially in patients without mutation in common genes or regions (66,67).

Therapeutic options for FH patients:

Since the 1990s, Statins (HMG-CoA reductase inhibitors) are the first-line treatment in most of FH patients (68). Nonetheless, many FH patients cannot get to the normal LDL-C levels with statins consumption; especially homozygous FH patients are often unresponsive to statins (69). Many efforts to achieve effective treatments for this group were conducted. Depends on patient's mutations, several new classes of pharmacotherapy and novel strategies exist to obtain greater LDL-C reduction. Some obtained results are presented here.

- ***Current therapies:***

I. Statins: statins can block the conversion of HMG-CoA into mevalonic acid, the precursor of cholesterol.As a consequence cholesterol synthesis is decreased; the expression of the LDL receptors in the liver isupregulated to enhance uptake and catabolism of LDL particles. *LDLR* activity is increased so, LDL-C in plasma is reduced.

***II. Ezetimibe*:** Ezetimibe is a cholesterol absorption inhibitor that prevents the absorption of cholesterol and plant sterols by inhibiting the passage of sterol of dietary and biliary origin across the intestinal wall.

III. Bile acid sequestrants (colesevelam, colestipol, and cholestyramine): these drugs can decrease the hepatocyte cholesterol content. This decrease results in an up-regulation of the *LDLR*.

IV. Nicotinic acids (niacin): niacin by an unclear mechanism reduces VLDL synthesis, which is affected LDL-C and increased HDL-C (70).

V. LDL apheresis: LDL apheresis can filter LDL particles from blood circulation through binding to dextran sulfate or heparin. Studies have shown that itresulting in an LDL-C reduction approximately 55%-75%. It's safe and beneficial especially for homozygote FH patient (71,72).

- ***Novel therapies:***

I. ApoB synthesis inhibitors (mipomersen): mipomersen is a second-generation antisense oligonucleotide (ASO). It is designed to target human apolipoprotein B *(ApoB)-100*. Mipomersen silences *ApoB* mRNA in liver, thereby reducing hepatic *ApoB-100*, total cholesterol and LDL-cholesterol in plasma. Mipomersen can decrease *ApoB*, LDL-cholesterol and lipoprotein (a) in patients with heterozygous and homozygous FH(73,74).

II. PCSK9 inhibitors: *PCSK9* inhibitors, including antisense oligonucleotides, short-interfering RNA and antibodies are used for lowering plasma LDL-cholesterol and improving the LDL-cholesterol-lowering ability of statins. However, they need some residual LDL-receptor function; so this approach is limited to heterozygous FH and

homozygotes with reduced LDL-receptor function (75-78).

III. Microsomal triglyceride transfer protein (MTTP) inhibitors: MTTP is an endosomal protein present in hepatocytes and enterocytes and plays a fundamental role in the assembly and release of *ApoB* containing lipoproteins, including LDL-C, TGs, phospholipids and cholesterol esters. There by MTTP inhibitors such as lomitapide interfere in the assembly of plasma lipoproteins in liver and reduce associated plasma lipid levels (79-82).

IV. Cholesterol ester transfer protein (CETP) inhibitors: CETP promote the exchange of cholesteryl esters from HDL to LDL and other lipoproteins. There by inhibition of this process increases HDL-C levels and reduces LDL-C levels (83,84). Four classes of CETP inhibitors are approved for FH patients until now: anacetrapib, dalcetrapib, evacetrapib and torcetrapib (4,85,86).

V. Apical sodium dependent bile acid transporter (ASBT) inhibitors: This transporter involves in reabsorption of bile acids and facilitates their return to the liver by the portal vein. Inhibition of ASBT can decrease bile acid reabsorption.ASBT inhibitors would be very useful in combination with either statin or Ezetimibe (87).

VI. Thyroid mimetics (eprotirome): Thyroid hormones involved in some important processes including lipid metabolism and control of energy consumption (88). New agonists to the thyroid receptor have been developed for LDL-C reduction. They act by stimulation of bile acid synthesisand stimulation of biliary excretion (89-91).

VII. Liver-directed somatic gene therapy: For first time hepatocyte transplantation (HTx) in FH was done by using ex vivo autologous hepatocytes transfected with recombinant retroviral *LDLR*. The recipient (homozygous FH) demonstrated modest improvements (30%) in LDL/HDL levels(92). Other efforts are underway to improve this procedure.

VIII. Liver transplantation: Recent reports of successful liver transplant done for the treatment of HoFH indicate high efficacy and safety for this option but According to the infrequency of liver donor and complexities of the transplant and post-transplant management this is not a popular choice for treatment (93,94).

Conclusion

FH is an inherited heterogeneous common disease that is associated with premature cardiovascular complications. There are over 13 million people in all over the world suffer from this condition. The comprehension of lipid biology during the last decade has led to the identification of novel strategies to inhibit specific pathways in hyperlipidemia. Hence, this knowledge can reduce the risk of cardiovascular problems and mortality in FH patients by help to better disease control and treatment. To apply appropriate screening strategies and timely initiation of prospertherapies, causative mutations should be identified in each society, and then pharmaco therapies and novel strategies can be utilized according to them.

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