Familial hypercholesterolemia: the Dutch approach

Huijgen, R.

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: https://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Chapter 1

General introduction and thesis outline

Roeland Huijgen, Maud Vissers, Barbara Hutten, John Kastelein

Adapted from:
Roeland Huijgen. Maud N. Vissers, Joep C. Defesche, Peter J. Lansberg,
John J.P. Kastelein, Barbara A. Hutten

INTRODUCTION

Familial hypercholesterolemia (FH) is the most common monogenetic disorder of lipid metabolism, with an average prevalence of heterozygous FH of 1 in 500 individuals.1 In 1939, the Norwegian internist Carl Müller was one of the first to describe FH as a clinical entity that is characterized by the familial expression of xanthomatosis, hypercholesterolemia, and heart disease. Later studies revealed the autosomal dominant inheritance of FH and, more than thirty years later, Michael Brown and Joseph Goldstein elucidated the underlying molecular defect. In 1985, they won the Nobel Prize for their discovery of a single gene responsible for the expression of a specific low-density lipoprotein cholesterol (LDL-C) receptor (LDLR).2

The clinical characteristics of FH are most profound in homozygous patients but they are often also evidently present in heterozygous patients. LDL-C is elevated about tenfold in homozygous- and threefold in heterozygous patients. The high LDL-C levels lead to deposition of cholesterol in various tissues, expressed as tendon xanthomas, xanthelasmas, and arcus cornealis. Accumulation of cholesterol in the arterial walls finally leads to premature atherosclerotic cardiovascular disease (CVD).3 In untreated heterozygous patients, about 50% of males and 30% of females develop CVD before the age of 60,3 whereas homozygous patients suffer from CVD events as early as in the second decade of life.1

Early detection of FH is essential in order to prevent or postpone cardiovascular sequelae by preventive measures such as pharmacological interventions. The last decade, several new pharmacological agents have been developed to optimize treatment for patients with FH. In this chapter, we will first consider several aspects of FH itself and we will briefly discuss the currently most prescribed treatment modalities. We will mainly focus on the more prevalent heterozygous FH that is of greater clinical significance than the relatively rare homozygous FH. Hence, the term “familial hypercholesterolemia” (FH) refers to the heterozygous form, unless otherwise noted.

Prevalence

FH affects on average 1 in 500 (0.2%) individuals,1 resulting in nearly ten million FH patients worldwide. Homozygous FH is rare, affecting 1 per million individuals. In some populations FH prevalence is higher because of genetic founder effects (i.e. the introduction of mutations by founders of this population).4 Apart from these founder populations, in most countries FH is caused by a diversity of mutations, with regional differences. This situation demonstrates the need for good diagnostic criteria.
for FH that are globally applicable. DNA-analysis technology for the detection of a plethora of mutations is available and makes molecular diagnosis in principle possible.

**FH causing mutations**

Specific mutations in the LDLR, APOB or PCSK9 genes result in an impaired clearance of LDL particles by hepatocytes and subsequently elevations in LDL-C plasma levels, characteristic for FH. The most prevalent underlying molecular defect of FH consists of one of the several mutations in the gene coding for the LDL receptor (LDLR) protein which is located on chromosome 19. Structural alterations in the LDLR result in an impaired clearance of LDL particles which subsequently lead to elevated LDL-C levels. More than 1000 different mutations in the LDLR gene or promoter region leading to an FH phenotype have hitherto been described. Mutations in the LDLR binding domain of the apolipoprotein B (APOB) are also known to cause high LDL-C levels. Only five hypercholesterolemic mutations of the APOB gene have been reported to cause familial defective apolipoprotein B (FDB). FDB is clinically indistinguishable from the classical form of FH, although it has a somewhat milder clinical phenotype with lower levels of LDL-C. Not all cases with FH can be explained on a molecular level by a mutation in the LDLR or APOB gene. A third locus for FH is located in a region on chromosome 1 encoding the proprotein convertase subtilisin/ kexin type 9 (PCSK9) protein. Variations in PCSK9 are a rare cause of nonLDLR/nonAPOB FH, and ten hypercholesterolemic mutations in PCSK9 were published (<3%). A prominent hypothesis states that gain-of-function mutations in PCSK9 cause hypercholesterolemia due to a process in which PCSK9 stimulates the redistribution from LDLR proteins from the cell membrane to lysosomes in hepatocytes and through this mechanism enhances degradation of LDLR.

The LDLR is abundantly present on the surface of liver cells where it specifically clears LDL-C from circulation. Normally, approximately 70% of LDL particles are cleared from circulation by the liver, and the activity of the LDLR in the liver is the most crucial regulator of LDL-C concentration in plasma. FH is the result of defective internalization of LDL particles into the hepatic cells via LDLR. The known genetic defects in LDLR, APOB, or PCSK9 hinder the LDLR mediated clearance of LDL-C from the plasma, as is depicted in Figure 1. The overall effect of these alterations is a decreased LDLR mediated clearance of LDL-C from the blood, leading to a higher LDL-C. Moreover, the decrease in the hepatic cholesterol pool stimulates cholesterol synthesis, resulting in the production of very low-density lipoprotein (VLDL) which further increases LDL-C levels.
Figure 1: LDL-Receptor mediated LDL-C uptake and altered pathway with FH causing defects in LDLR, APOB100 and PCSK9.

Panel 1 shows the LDLR mediated binding and uptake of LDL-C in the normal functioning hepatocyte, after which the LDL-C is metabolized and LDLR can be either recycled to the cell surface or is degraded. Panel 2 shows the effect of an LDLR mutation which can lead to decreased production or functionality or recycling of the LDLR. Panel 3 shows the effect of an FDB mutation in the binding region of the APOB100 protein, which results in impaired binding between APOB100 and LDLR. Panel 4 shows the mechanism of a PCSK9 gain-of-function mutation (PCSK9 gof), which is hypothesized to decrease the recycling of LDLR to the hepatocyte surface and increasing the degradation of the LDLR after LDL-C uptake has taken place. Reproduced from *Expert Review of Cardiovascular Therapy*, April 2008, Vol. 6, No. 4, Pages 567-581 with permission of Expert Reviews Ltd.
Diagnosis
A diagnosis is usually made on the basis of clinical features. The primary clinical diagnostic criteria for FH are an elevated LDL-C level, presence of tendon xanthomata in the patient or a first degree relative, and a pattern of autosomal dominant inheritance of premature coronary heart disease or hypercholesterolemia. Several tools for the clinical diagnosis of FH have been developed: the US MedPed (Make Early Diagnosis to Prevent Early Deaths) Program, The Simon Broome Register Group in the United Kingdom, and the Dutch Lipid Clinic Network. The MedPed Program uses cut points of total cholesterol levels based on age and family history to diagnose FH. The Simon Broome Register Group also uses cholesterol levels, but adds physical characteristics, molecular diagnosis, and family to the criteria. The presence of one or more combinations of these criteria lead to a “probable” or a “definite” FH diagnosis. The Dutch Lipid Clinic Network assigns scores for family history, clinical history, physical examination, LDL-C levels and DNA analysis. The overall score yields conclusions such as “definite”, “probable” or “possible” FH.

Although these diagnostic tools are useful for standardization of diagnosis, they do not necessarily result in consistent sensitivity and specificity. In the Danish population, it was demonstrated that when relying solely on biochemical data with cut-off values for total or LDL-C, 23% or 33% of genetically diagnosed FH patients would have been missed when using respectively the 90th or 95th percentiles of LDL-C for age and gender. Moreover, cholesterol levels vary with age and gender and are population specific. Therefore, the cut-off level for diagnosis of hypercholesterolemia should ideally be age, gender and population specific. However these lipid values are not always available for each specific group, in particular for children and adolescents.

Genetic analysis, i.e. the demonstration of a causative functional mutation, possibly provides the only unequivocal diagnosis and does so at an early age. This latter aspect is important because maximum health benefit can be obtained in FH if identification and treatment are commenced as early as possible as the World Health Organization has recommended.

Screening for FH
World Health Organization guidelines for conditions for which effective screening is worthwhile hold true for FH: it is an important public health problem, it can be accurately diagnosed, and effective treatment exists. Furthermore, effective primary prevention in patients with FH requires early diagnosis. Genetic analysis for FH in probands with a clinical diagnosis of FH and expansion within their families can unequivocally identify affected and non-affected persons within families. This
method of identifying FH has been applied in the UK,\textsuperscript{19} Norway,\textsuperscript{20} Spain,\textsuperscript{21} Iceland,\textsuperscript{22} and the Netherlands.\textsuperscript{23} The Dutch have the most extensive experience with genetic screening of relatives. Since 1994, cascade screening is the method of screening for FH in the nationwide program.\textsuperscript{23} The majority of newly identified mutation carriers were unaware of having FH before diagnosed with FH and only 38\% received lipid lowering treatment. Two years after diagnosis this figure improved dramatically and more than 86\% of the identified FH patients received lipid lowering treatment.\textsuperscript{24} The Dutch approach of cascade screening was demonstrated to be cost effective\textsuperscript{25} and recently the Ministry of Health has taken responsibility for coordinating the screening program to facilitate and monitor its expansion. They aim to identify all FH carriers -an estimated 40,000- in the Netherlands. To date almost 27,000 FH carriers have been identified by this screening program.

**Phenotypic expression of FH**

Although FH is a monogenic disorder, the phenotypic expression in terms of dyslipidemia, onset and severity of atherosclerotic disease varies considerably.\textsuperscript{26} There have been attempts to correlate the specific types of FH-causing mutations to the severity of CVD risk or dyslipidemia.\textsuperscript{26-29} In general, receptor negative alleles were found to be associated with a more elevated LDL-C and CVD risk than were receptor-defective alleles.\textsuperscript{26} Furthermore, $\textit{LDLR}$ mutations lead to a more severe phenotype than those in $\textit{APOB}$.\textsuperscript{9}

In the Netherlands, substantial numbers of several mutation carriers have been identified by the cascade screening program and therefore cardiovascular risk per mutation could be studied based on large numbers. Souverein and colleagues studied the specific influence of the 86 most prevalent $\textit{LDLR}$ mutations in the Netherlands on the onset of cardiovascular events. When they adjusted for LDL-C levels, the additive effect on CVD for each specific mutation varied non-significantly from the others. They concluded that the event free survival depends more on actual LDL-C levels caused by the mutation than on the type of mutation itself.\textsuperscript{28}

**Natural history of FH**

One of the first reports which demonstrated a significant association between coronary heart disease (CHD) and clinical FH was the study of Slack in the beginning of the second half of the 20\textsuperscript{th} century. Further studies uniformly demonstrated an increased burden of premature CHD and death associated with the presence of FH, with a lower incidence in Japan than in Western countries.\textsuperscript{30, 31} Moreover, a study of Slack showed that the probability of a first episode of ischemic heart disease for men and
women were 5% and 0% by the age of 30, 51% and 12% by the age of 50, and 85% and 57% by the age of 60, respectively. These findings suggest not only a difference in CHD risk between age groups of FH patients, but also between men and women. In general the onset of disease appears to be approximately 10 years earlier for men compared to women.

**Imaging of atherosclerosis in FH**

The progression of atherosclerotic disease can be visualized and measured in several ways. One of them is the measurement of intima-media thickness (IMT) of carotid and femoral arteries. In FH patients, the IMT have been extensively used as a surrogate endpoint to assess the effect of pharmacological intervention. Numerous studies have shown that the IMT constitutes a reliable marker for the risk of cardiovascular events. Therefore, the IMT is widely accepted as a validated surrogate marker for atherosclerotic disease.

De Groot and colleagues compared the IMT of 315 FH patients (age range 11 to 67 years; LDL-C 7.2 ± 1.8 mmol/L) with 118 unaffected subjects (age range 11 to 76 years; LDL-C 3.1 ± 0.8 mmol/L). The carotid IMT of 0.78 mm observed in the FH population at the age of 40 was similar to the IMT measured in the unaffected population at the age of 76. They concluded that the rate of IMT progression was significantly higher in the group of FH patients compared to controls (0.009 and 0.004 mm/year, respectively). Moreover, a comparison of the IMT between children with FH and their non-affected siblings revealed a significant difference from the age of 12, suggesting that the atherosclerotic process has already been initiated in childhood.

**Treatment of FH**

High levels of LDL-C have been consistently shown to be associated with CHD risk. The link between LDL-C lowering and reduction of CVD risk is now clearly established, leading to "the lower, the better" paradigm in hypercholesterolemia management. In general, FH patients are advised to adhere to a healthy lifestyle including a strict diet, frequent physical activity and no smoking. However, these lifestyle modifications hardly ever result in acceptable LDL-C levels and therefore the vast majority of patients with FH depend on pharmacological interventions to lower LDL-C. Since acceptable levels can not be reached in most FH patients, one should attempt to reach levels as low as possible by using – a combination of – powerful therapy.
Statins
Since their introduction in 1987, hydroxymethylglutaryl coenzyme A reductase (HMG CoA) inhibitors (statins) have gradually assumed a central role in the primary and secondary prevention of CVD and are currently considered the most effective drugs lowering circulating LDL-C levels. As a consequence, statin induced LDL-C reduction has become the cornerstone of current treatment guidelines for cardiovascular prevention.

Although there were no randomized placebo-controlled clinical trials specifically in patients with FH, the clinical efficacy of statins in reducing CVD was highlighted in the large Simon Broome cohort with clinical FH subjects. The time frame of this study, which overlies the introduction of statins, allowed observations and comparison between treated and non-treated FH patients in 1999: data suggest that treatment is effective in lowering the risk of CHD in patients with clinical FH. This was shown by the relative risk for coronary mortality which declined from 1992 onwards, and was especially pronounced in the younger age group (age 20-59 years).

Thus, statin treatment is currently the most effective treatment available and has been demonstrated to be effective in reducing CHD risk in FH, shown for standardized mortality ratios, as well as for cardiovascular events in an analysis in a large cohort of Dutch FH patients. However, a post-marketing survey performed in France on myalgia in patients using high dose statin treatment indicates that 10.5% of the patients report muscular symptoms shortly after initiation of treatment. These and other effects may preclude some patients from chronic statin treatment. Moreover, treatment of FH often requires therapy modalities that lower LDL-C over 50%. Therefore either a combination of statins with other drugs or high dose statin therapy is frequently required.

Other treatment options
The current and future treatment options beyond statin treatment are discussed in detail in chapter 16. The currently available and widely prescribed treatment options for FH patients will be briefly mentioned below. The addition of ezetimibe can enhance the LDL-C lowering effect of even highly potent statins and the combination is often used. It is, therefore, the second most prescribed agent for patients not reaching acceptable LDL-C targets with statin treatment. Ezetimibe specifically impedes the transport of cholesterol and phytosterols by blocking the Niemann-Pick C1-like 1 (NPC1L1) transporter in enterocytes. Ezetimibe hereby inhibits cholesterol transport through the enterocyte, which results in a reduced cholesterol
transport to the liver via chylomicrons. Through this mechanism, ezetimibe reduces the absorption of dietary and biliary cholesterol by preventing its transport through the intestinal wall. This induces a compensatory increase in LDL receptor expression, increasing the clearance of LDL-C from the plasma and reducing LDL-C levels. Ezetimibe can be easily added to statin therapy since both treatments act via separate pathways. Combination therapy of ezetimibe with several statins resulted in 12-23% incremental decrease in LDL-C when compared to statin treatment alone. However, it should be noted that the addition of ezetimibe has not yet been proven to reduce CHD risk.

Upregulation of bile acid biosynthesis can be used as a means of treating hypercholesterolemia. Interruption of the enterohepatic circulation of bile by preventing bile reabsorption by bile acid sequestrants or intestinal bile acid transporter inhibition leads to a rapid upregulation of hepatic bile acid synthesis. As a consequence of the diminished return of bile acids to the liver, bile acid synthesis from cholesterol by cholesterol 7 alpha hydroxylase (CYP7A1) is increased in order to maintain constant bile acid levels. The decrease in cholesterol levels due to conversion to bile acids finally leads to decreased LDL-C levels, mediated by increased hepatic LDLR expression.

Bile acid sequestrants lower cholesterol levels by binding bile acids in the intestine. Colestyramine, colestipol and colesevelam are the most widely used agents in this class of cholesterol lowering drugs, which reduce LDL-C by 10-30%. Colestyramine was the first lipid lowering drug to show a survival benefit in hypercholesterolemic patients in a large placebo-controlled trial. Colestyramine and colestipol are associated with gastrointestinal side effects, in particular constipation and flatulence, making long-term compliance poor. Colesevelam – a newly engineered bile acid sequestrant – has a greater potency to bind bile acids per gram of product and may therefore provide a much better tolerability profile than the other sequestrants. If combined with ezetimibe, it reduced LDL-C by an additional 11%.

Foods enriched with plant sterol or stanols can also inhibit the uptake of dietary and biliary cholesterol from the small intestine. Plant sterols and stanols presumably displace cholesterol from mixed micelles and thereby reduce intestinal absorption, but the exact mechanism is unknown. Abundant evidence shows that consuming 2 g/d of plant sterols and stanols reduce LDL-C by about 10%. Several studies in children with FH showed a modest reduction in LDL-C of 9-18% from plant stanol enriched margarine, suggesting that this is an effective and safe remedy for treating FH patients in childhood. Consumption of foods enriched with plant sterols or stanols is generally recognized as safe. Based on the current evidence, the NCEP-
ATPIII reports recommend a diet designed for maximal LDL-C lowering including foods that are enriched with plant sterols/stanol (2g/day).40

The future treatment options beyond above mentioned treatment regimens are discussed in detail in chapter 16. These various options range from LDL-C apheresis, LDLR gene-therapy, antisense therapy to APOB or PCSK9, to HDL-C raising agents. In the next few years, phase III trials will evaluate the efficacy and safety of the addition of new agents to currently applied combination therapy. These developments will undoubtedly result in the possibility for more effective treatment that fits the individual FH patient.

In summary, FH is a prevalent inherited disorder of the lipoprotein metabolism which leads to severely elevated levels of LDL-C and premature atherosclerosis. Genetic screening is effective in identifying FH in young subjects. Cholesterol lowering treatment in FH patients can prevent or delay the onset of cardiovascular disease and premature death. Not surprisingly, global initiatives are ongoing to screen and identify FH carriers in order to start therapy early for primary prevention. Statins are considered the first choice of treatment. All in all, with several new therapeutic options in advanced stages of development, this will undoubtedly result in the possibility for more effective treatment that fits the individual FH patient.

Thesis outline

This thesis is divided into four parts;

Part 1 focuses on the genetic diagnosis of FH and the required steps to confirm that the specific mutation identified is indeed pathogenic. In Chapter 2 we investigate a large pediatric population with an unequivocal autosomal dominant hypercholesterolemia (ADH) phenotype to assess the molecular basis of hereditary hypercholesterolemia and to define the percentage of individuals with unexplained dyslipidemia. Chapter 3 further elaborates on the clinical FH patients from Chapter 2 in whom no causal mutation was found. We perform whole exome sequencing in selected individuals within a family with a convincing ADH pattern. Accordingly, we describe the discovery of a pathogenic mutation in a region in the Apolipoprotein B gene that was previously not assumed to cause hypercholesterolemia. Chapter 4 presents a prospective study on the accuracy of genetic FH test results, based on a comparison of test results from the reference DNA laboratory with those from a counter-expertise laboratory. In Chapter 5 we design criteria to detect non-pathogenic mutations, based on lipid levels and cholesterol lowering medication use. Subsequently, the proposed criteria are applied to known pathogenic and non-
pathogenic LDLR and AP0B sequence variants from the FH screening registry. Last, these criteria are applied to assumed pathogenic prevalent mutations in order to discover non-pathogenicity among those sequence variants. In Chapter 6 we assess whether true pathogenic mutations are indeed associated with the occurrence of coronary artery disease (CAD) when compared to non-functional variants. Chapter 7 provides the distribution of the most prevalent pathogenic FH mutations in the Netherlands.

Part 2 of this thesis focuses on the variable phenotype of patients with confirmed FH mutations. We evaluate several factors which may explain why some mutation carriers do not express a severe FH phenotype. In Chapter 8 we describe our efforts to highlight why we observe such a large proportion of mutation carriers not expressing an FH phenotype. We focus on the effect of mutation severity and selective medication use in mutation carriers on the overlap in LDL-C levels between carriers and non-carriers. Chapter 9 presents the results of a large cross-sectional study evaluating the atherosclerotic burden among FH patients without high LDL-C levels, compared to classical FH patients with hypercholesterolemia and controls. We specifically aimed to discover whether individuals without a penetrant FH mutation are at increased CAD risk or not. Chapter 10 investigates the relationship between plasma levels of proprotein convertase subtilisin kexin type 9 (PCSK9) and LDL-C phenotype in FH patients and controls. PCSK9 is a natural inhibitor of the LDL-receptor and we hypothesized that low plasma levels of PCSK9 would associate with a less severe FH phenotype. This hypothesis is tested in the study population from the cross-sectional study described in Chapter 9. In Chapter 11 we sequence the genes encoding AP0B, PCSK9 and ANGPTL3 to address whether monogenic dominant loss-of-function mutations in those genes underlie a paradoxical phenotype in carriers of an FH mutation with normal cholesterol levels. Specific mutations in AP0B, PCSK9 and ANGPTL3 are known to result in a hypobetalipoproteinemia phenotype. Therefore, we hypothesize that carriership of such loss of function mutations would associate with a less severe FH phenotype in individuals with a concurrent pathogenic FH mutation. In Chapter 12 we describe two individuals with an extreme FH phenotype and focus on the concurrent mutations in the LDLR and 7-alpha-hydroxylase genes that explained their extraordinary features. In Chapter 13 we determine coagulation factor VIII (FVIII) levels in individuals that underwent cascade screening for genetic FH. FVIII plays a crucial role in the coagulation cascade, but the factors determining its level, are hitherto largely unknown. Previous studies have recently suggested a role for the LDLR in the regulation of this coagulation factor. Accordingly, we
hypothesized that patients with FH would have higher FVIII levels than their unaffected relatives. We test this hypothesis in the study population from the cross-sectional study described in Chapter 9.

In Part 3 of this thesis we focus on access to life insurance after genetic FH diagnosis. Chapter 14 reviews the reported problems with access to insurance after genetic FH. In addition, it describes the advent of the guidelines for insurance companies that were designed to reduce genetic discrimination in our country. In Chapter 15 we describe our efforts to compare life insurance acceptance rates before and after FH diagnosis and before and after the guidelines, as described in Chapter 14, were issued. We hypothesize that access to life insurance has improved for FH patients after the guidelines were issued.

In Part 4 of this thesis we focus on treatment of FH patients, in particular the current treatment options, the need for additional treatment modalities and two novel treatment strategies. Chapter 16 provides a review of current and future treatment modalities for FH. In Chapter 17, we take a close look at cholesterol lowering medication use two years after genetic FH diagnosis. In Chapter 18, we describe our effort to calculate the effectiveness of the genetic screening program in preventing CAD. This calculation is based on the medication use after genetic diagnosis, as described in Chapter 17, and the reported CAD risk reduction through statin treatment from a large Dutch clinically diagnosed FH cohort. Chapter 19 investigates the LDL-C goal target achievement in relation to the prescribed treatment regimen among FH patients referred to five large outpatient lipid clinics. In Chapter 20 we examine the efficacy and tolerability of the bile acid sequestrant colesevelam in patients with FH with an LDL-C level above target despite a maximally tolerated regimen of a statin and ezetimibe, in a 12-week randomized controlled trial. To predict the effect of inhibiting PCSK9 on CAD incidence in statin treated patients, in Chapter 21 we performed a post-hoc analysis of theTreating to New Targets(TNT) study. The TNT-study is a randomized trial that compared the efficacy of high- versus low-dose atorvastatin. To this end, we measured PCSK9 plasma levels among patients with stable CAD treated with atorvastatin 10 mg daily, who were randomized to either continue with 10 mg or be up-titrated to 80 mg of atorvastatin, and followed during 5 years for cardiovascular events.
REFERENCES


5. www.ucl.ac.uk/fh, last visited July 2012


General introduction and thesis outline


