Genetics and therapy of familial hypercholesterolemia

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CHAPTER 1

General introduction

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Cardiovascular disease
Cardiovascular disease (CVD) is the most important clinical consequence of atherosclerosis and is currently the major cause of morbidity and mortality in the Western world. World Health Organization (WHO) statistics illustrate that this trend will continue into this new millennium, with an even more impressive contribution to disease statistics.\(^1\)

It was probably Leonardo da Vinci (1452-1519) who first recognized the macroscopic changes of atherosclerotic vascular disease. While sketching the arterial lesions at autopsy of an elderly man he attributed the vascular changes to an excessive nutrition from the blood. Much later, in 1913, the Russian researcher and physician Anitschkow confirmed these findings in animals. He fed rabbits with egg-yolk and demonstrated that increased serum cholesterol accelerated atheroma formation in the walls of the aorta. Development of atherogenesis is characterized by different stages, which are evident in studies in animals as well as in humans who died from other causes. The first stage of atherogenesis comprises fatty streak formation where foam cells accumulate in the subendothelial space. These foam cells undergo necrosis and become covered by a fibrous cap of smooth muscle cells, that have proliferated together with different structural elements including collagen. This comprises the fibrous plaque. Patients with these plaques will develop clinical symptoms only if blood flow downstream of this plaque is insufficient which will lead to ischemia (stable angina or claudication intermittens) or conversely if the plaque ruptures and thrombosis follows with complete obstruction of the arterial blood flow resulting in infarction (acute myocardial infarction or cerebral infarction).

Clearly, both genetic and environmental factors play a major role in the causation of CVD. Genes contribute to coronary risk factors such as lipoprotein production and clearance, blood pressure dynamics and glucose metabolism, but they also contribute to an individuals' response to environmental challenges such as diet, drugs and tobacco. For example, studies have shown that some patients respond better to a change in dietary cholesterol than other others\(^2\) and that patients can exhibit a smoking-dependent risk of coronary artery disease associated with a polymorphism of the endothelial nitric oxide synthase gene.\(^3\)

Furthermore, our genes play a significant role in determining altered predisposition to CVD over time, in the way we respond to obesity and diabetes mellitus. Therefore,
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genetic testing is likely to be more predictive of our predisposition to CVD than current testing of known risk factors. Especially in young patients with CVD, genetic influences will predominate over the environmental influences. The actual mechanisms that predispose people to CVD have until recently remained undetermined. However, recently research has led to a greater understanding of the genetic basis of atherosclerosis. Disturbances in lipids, changes in blood vessel wall and macrophages and the thrombolytic system contribute to this susceptibility.

Familial hypercholesterolemia

In 1873, initially, familial hypercholesterolemia (FH) was recognized as a skin disease with xanthomas and xanthelasmas. Many years later, in 1938, Müller found that FH was associated with a high incidence of premature atherosclerosis, resulting in coronary, cerebral or peripheral vascular disease. It was Khachadurian who demonstrated in 1963 the autosomal dominant mode of inheritance. The heterozygous and homozygous forms of FH, and their distinctive clinical expression, were also recognized at that time. Later, it became evident that the increased level of plasma low-density lipoprotein (LDL)-cholesterol was the hallmark of the disease. After this observation, the role of the LDL-receptor in clearing LDL-cholesterol became clear. In 1953 Watson and Crick discovered the structure of DNA. The knowledge of the DNA structure in combination with the autosomal inheritance of FH and the role of the LDL-receptor finally led to the discovery of the underlying molecular defect of FH consisting of mutations in the LDL-receptor gene. This observation by Brown and Goldstein led to the Medical Nobel Prize in 1985.

The LDL-receptor gene locus is located on the short arm of chromosome 19 and spans 45 kb with 18 exons and 17 intervening introns, encoding a mature protein of 839 amino-acids. At present, 920 mutations in this gene are known to underlie FH and still new mutations are identified regularly. The frequency of FH in the population of Europe and North America averages about one in 500. However, in some regions of the world the prevalence is much higher owing to founder effects that occur when a mutant allele is introduced within a population, in which physical isolation gives rise to limited mixture with persons of other origins. An increased frequency of consanguineous marriages and large numbers of offspring is often encountered in this situation. Moreover FH is a disease where patients usually reach adult age and can therefore create a next affected generation. The highest frequency of FH occurs in South Africa, where one in 100 white Afrikaners have the disorder.
The V408M mutation in exon 9, which is found in 15% of FH cases in Afrikaners, is a fairly common mutation in Dutch FH patients (1.6%) and has not been described in persons of other ancestry. In addition, haplotype analysis that showed that at least part of the Dutch FH patients carrying the V408M mutation in exon 9 shared the same haplotype with Afrikaner patients. These findings support the hypothesis that the V408M mutation originated in the Netherlands and was introduced in the Afrikaner population in the 17th century by Dutch settlers.10

The estimated FH population in the Netherlands is 35,000 persons, but case-finding research in the northern part of the country showed an even higher prevalence of 1:400 11. Some parts in the Netherlands show an especially high prevalence of a specific mutation, but mostly many different mutations can be found. More than 150 different mutations have been identified in the Netherlands.12 In contrast, in Iceland, only 3 different mutations explain almost all FH patients due to the homogeneity of the Icelandic people.13

In general, mutations consist of point mutations, small insertions and deletions and large rearrangements. Based on the effect of the mutation on the LDL-receptor protein function, LDL receptor mutations can be divided into five classes. Class 1 mutations fail to produce receptor protein (null-alleles). Class 2 mutations encode proteins that are blocked, either partially or completely, in transport between the ER and the Golgi complex (transport-defective alleles). Class 2 mutations can be subclassified into class 2A and class 2B mutations. Class 2A mutations produce proteins that are transported at a detectable, but reduced rate. Class 2B mutations encode for proteins that are characterized by slow transports to the Golgi. Class 3 mutations encode proteins that are synthesized and transported to the cell surface, but fail to bind LDL normally (binding-defective alleles). Class 4 mutations have a normal synthesis of the LDL receptor protein and normal binding of LDL, but clustering in coated pits and internalization of the receptor complex does not take place (defective-alleles). These mutated receptors are synthesized normally, folding and transport are normal, but clustering in coated pits is impossible (class 4A) and sometimes the receptors are even secreted after they have reached the cell surface (class 4B). Class 5 mutations are mutations in the domain that mediates the acid-dependent dissociation of receptor and ligand in the endosome, an essential event for receptor recycling (recycling-defective alleles).14,15

Apart from mutations in the LDL-receptor, a disease named familial defective apolipoprotein B (FDB) is clinically identical to FH. In patients with FDB a mutation
in the ligand binding domain of the apolipoprotein B gene causes a defective binding of LDL particles to the LDL-receptor. Only a molecular diagnosis can differentiate between FH and FDB patients.\textsuperscript{16}

In FH, due to an impaired function of the LDL-receptor, LDL-cholesterol is insufficiently taken up by the receptor in the liver and LDL-cholesterol concentration in plasma will rise to approximately twice-normal levels. This leads to excessive deposition of cholesterol in the arterial wall and peripheral tissues, leading to accelerated atherosclerosis and premature cardiovascular disease. Coronary artery disease may manifest in the fourth or even third decade of life. Typically, approximately 45\% of male and 20\% of female patients have documented coronary artery disease (CAD) by the age of 50.\textsuperscript{17}

FH is characterised by large differences in the expression of the clinical phenotype. Even FH patients with identical mutations and LDL-C levels can have significant differences in clinical outcome, such as widely different ages of onset of CVD.\textsuperscript{18} It is hypothesised that both genetic and environmental factors contribute to this phenomenon. For example, FH patients living in China have significantly lower LDL-C levels compared to family members who immigrated to Canada. Furthermore, neither xanthomas nor premature CAD were seen in rural Chinese patients but did occur in Canadian relatives.\textsuperscript{19} However, solid data addressing the contribution of environmental and genetic factors to the variable phenotypic expression of FH are scarce.

Recently, new additional risk factors have been identified in populations other than FH patients. Among these are homocysteine levels, remnant-like particles levels, bilirubin levels and many fibrinolytic and coagulation parameters. The strategy to investigate possible risk factors involves a combination of genetic and biochemical studies of candidate genes. These candidate genes are likely to affect lipoprotein phenotype such as genes coding for apolipoproteins, enzymes and lipoprotein receptors. Many genes encoding proteins critical in lipoprotein metabolism have been cloned and sequenced. Among these are lipoprotein lipase (LPL), apolipoprotein E, microsomal triglyceride transfer protein and cholesteryl ester transfer protein.

FH as a disease can be used as a model to test these candidate genes in the pathogenesis of atherosclerosis because the disease carries such a high a priori risk for CVD. In order to better quantify the atherosclerotic process in these studies we have developed surrogate markers, such as intima-media thickness of the carotid and femoral arteries.
**Intima-media thickness**

A well validated non-invasive method for the assessment of atherosclerosis is the intima-media thickness (IMT), as measured by B-mode ultrasonography in the carotid and femoral arteries. The edges of the lumen-intima and media-adventitia ultrasound interfaces of the posterior artery walls represent the boundaries of the intima-media complex. The distance between the interfaces is therefore called intima-media thickness (IMT) (figure 1).^{20,21}

**Figure 1.** B-mode (IMT) ultrasound technique.

Increased intima media thickness (IMT) in a FH patient. The distance between the left and right arrow indicates the IMT-complex (figure 1A). The distance between the leading ultrasound interfaces of the double-line pattern of the arterial far wall, the intima-media thickness (IMT) is directly related to the thickness of the histological defined intima-media complex (figure 1B).

IMT is associated with age and cardiovascular risk factors such as LDL-C, blood pressure and smoking.^{22,23} Moreover, intervention trials have shown that by reducing risk factors, such as LDL-C levels, progression of atherosclerosis was inhibited,^{26} or even led to regression of this process.^{27} IMT measurements are therefore now widely accepted as a standardized and validated surrogate marker for atherosclerotic vascular disease.^{26,30}

We were the first to demonstrate that intensive lipid lowering with 80 mg atorvastatin resulted in the actual regression of carotid IMT in the majority of FH patients, and that 40 mg of simvastatin only led to less progression of vascular wall IMT.^{27}
THE EXPRESS STUDY: A MULTI-CENTER STUDY
(Examination of Probands and Relatives in Statin Studies with Familial Hypercholesterolemia)

All participating sites in the ExPRESS study were members of the MedPed (Make Early Diagnosis to Prevent Early Death) program. This network has been created to trace family members of FH patients who are at increased risk of developing cardiovascular disease. MedPed is an international organisation with more than 34 countries currently participating. The importance of the efficacy of selective screening in families with FH has been recognized internationally and endorsed by the World Health Organisation. Also in the Netherlands a foundation for tracing hereditary hypercholesterolemia (StOEH) was founded to identify all FH patients in the Netherlands by screening family members of a FH patient with an identified mutation in the LDL-receptor.

Mutations in the LDL-receptor can be divided in receptor-negative (null-allele) or receptor-defective mutations. In receptor-defective mutations the activity is somewhat decreased, whereas it is extremely low (≤5%) or absent in receptor-negative mutations. In the past, some studies reported different responses to statin therapy for mutations divided according to the LDL-receptor activity. However, most of these studies were performed in small study populations with very few different mutations. These findings served as the background for the conduction of a multi-center study in the Netherlands: the ExPRESS study (Examination of Probands and Relatives in Expanded Statin Studies). For this open label multicenter study FH patients were recruited from 37 Lipid Clinics in the Netherlands. Patients were included if they had either a molecular diagnosis for FH or were diagnosed with definite FH and had to have 6 or more points, according to an algorithm (to allow standardization of the diagnosis of FH based on clinical findings, personal and familial clinical history and biochemical parameters). After a washout period of 6 weeks, patients were started on monotherapy with simvastatin 80 mg, one tablet once daily, for 2 years. No other lipid lowering medication was allowed. Medical history, physical examination and additional risk factors for cardiovascular disease as well as laboratory analysis of lipid and lipoprotein levels and routine safety parameters were obtained in all patients. The biochemical analyses of lipid levels and safety parameters were performed in the hospitals themselves at each of 8 clinic visits (at weeks: -6, 1, 6, 12, 24, years: 1, 1½ and 2) and were standardized by a virtual central laboratory. The DNA analyses were performed in the Academic Medical Center in Amsterdam.
OUTLINE OF THE THESIS

The purpose of the thesis was to investigate the relationship between different LDL-receptor mutations and the response to treatment with simvastatin 80 mg. For this purpose many different Lipid Clinics in the Netherlands were asked to participate to gather a diverse FH population. All patients who were on lipid-lowering therapy underwent a wash-out period of 6 weeks prior to initiation of simvastatin 80 mg.

First, a general introduction of atherosclerosis, familial hypercholesterolemia and intima-media thickness measurements is provided in Chapter 1. The collection of a large group of more than 500 well defined FH patients provided the opportunity to describe possible other known risk factors apart from elevated LDL-cholesterol levels at baseline as described in Chapter 2. Furthermore, a newly identified risk factor could be examined in FH patients. Low concentrations of bilirubin are associated with an increased risk for CAD both in retrospective and prospective studies. Possibly, bilirubin exerts its effect through protection of LDL from oxidation. In Chapter 3 we examined whether low bilirubin might also be a risk factor for CVD in patients with familial hypercholesterolemia.

In Chapter 4 safety and efficacy of simvastatin 80 mg over a period of two years is described in FH patients. This is the first long term safety and efficacy study with simvastatin 80 mg. In Chapter 5 the frequency of abnormal TG and HDL-cholesterol levels in patients with FH was evaluated and therapeutic response at different baseline levels of these lipoproteins after one-year of statin therapy was assessed.

Remnant lipoproteins (RLP-C) are considered important in atherogenesis. Hence, in Chapter 6 baseline RLP-C levels and the effect of statin therapy in patients with FH are described. Elevated RLP-C levels have been associated with the presence and progression of atherosclerotic disease and their presence in FH patients has been proposed but never established in a large cohort, or their response to statin therapy.

In Chapter 7 we investigated whether high dose simvastatin therapy could reduce carotid and femoral artery IMT in patients with familial hypercholesterolemia (FH) in order to prevent CVD. The primary endpoint was the change in mm of the mean combined far wall IMT of predefined carotid and femoral arterial segments at two years. Hyperhomocysteinemia is associated with atherosclerosis, but a causal relationship is still vehemently debated. In Chapter 8 the relation between baseline plasma homocysteine levels and CVD, IMT measurements and the methylenetetrahydrofolate reductase (MTHFR) genotype is described.
The beneficial effect of statins on cardiovascular morbidity and mortality is hypothesized not only to result from LDL- cholesterol reduction but also from mechanisms that modify hemostasis. In Chapter 9 we evaluate whether high dose simvastatin therapy could modulate markers of coagulation and fibrinolysis and to evaluate whether changes in these markers after two years of statin therapy were associated with changes in IMT of the arterial wall as marker for atherosclerosis progression.

Formerly, some studies reported different responses to statin therapy for mutations divided according to the LDL-receptor activity. In Chapter 10 the response to statin treatment for a total of 71 different mutations in 343 patients divided into receptor activity ≤5% and receptor activity >5% is presented.

REFERENCES

Chapter 1
