The spectrum of premature atherosclerosis: from single gene to complex genetic disorder
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General introduction
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Atherosclerotic vascular disease, such as coronary artery disease, ischemic stroke and peripheral arterial disease represent a major health problem that is associated with high rates of morbidity and mortality, particularly in Western societies. The atherosclerotic process begins in childhood but, in general, does not reach the clinical horizon until after the fifth decade of life, at which point the best opportunities for prevention have been lost. Therefore, it is important to identify individuals at high risk for cardiovascular disease (CVD) early in life and institute adequate measurements for primary prevention.

Numerous conditions are known to predispose to CVD, commonly referred to as risk factors. Generally accepted risk factors include dyslipidaemia, hypertension, diabetes, obesity, metabolic syndrome, cigarette smoking, physical inactivity, male gender, menopause, hyperhomocysteinemia, and a positive family history of CVD. This listing serves to show that there is no single cause for CVD, and that atherosclerosis should be perceived as a complex or multifactorial trait. In addition to the well-established, in part “external”, biological risk factors, several lines of evidence have established beyond doubt the pivotal role of genetic risk factors in influencing CVD risk. In fact, it is becoming increasingly clear that the analysis of genetic and biological risk factors, together with lifestyle and environmental factors may contribute significantly to our understanding of the predisposition to CVD.

Most common diseases, like CVD, represent quantitative traits that are caused by interactions among genes and between genes and the environment. An important objective in research is to unravel the mechanisms of how genetic and environmental factors combine or negate to cause the clinical sequelae.

Premature atherosclerosis resulting in CVD in young patients is rare and has some characteristics that are different from those in elderly patients. The most important issue is the fact that premature cardiovascular disease shows a strong familial aggregation, indicating a significantly heritable component. Therefore, studies for the elucidation of genetic risk factors for CVD can best be performed in a young study population.

In some diseases like Familial Hypercholesterolemia (FH), the genetic factor is clearly defined. FH can be considered as a single gene disease, the LDL-cholesterol receptor gene or the Apo-B 100 gene is mutated and LDL-cholesterol markedly increased. Due to the high levels of LDL-cholesterol, patients with FH have an increased risk of premature CVD.
In most cases of premature familial CVD, however, we do not yet understand the underlying mechanism. Since atherosclerosis is a multifactorial and a complex genetic disorder a variety of genes are involved in most cases of familial premature CVD. (Figuur 1)\(^7\)

This theses focuses on premature atherosclerosis, the influence of genetic risk factors, and the impact of treatment on cardiovascular risk.

**Background and outline of part I**

Heterozygous Familial Hypercholesterolaemia (FH) is an autosomal dominant disorder with a frequency of about 1 in every 500 individuals. The underlying defect is a mutation in the gene encoding for the LDL receptor or for apolipoprotein B-100. As a result, LDL-cholesterol is insufficiently taken up by the receptors in the liver and LDL-cholesterol concentrations in plasma will rise to approximately twice normal levels. In most patients, there is excessive deposition of cholesterol in peripheral tissues leading to specific tendon xanthomas and to accelerated atherosclerosis and premature CVD. Heart disease may manifest in the fourth or even third decade of life. Since the introduction of HMG-CoA reductase inhibitors (statins), substantial lowering of LDL-cholesterol can be achieved, resulting in beneficial effects on cardiac morbidity and mortality. Aggressive treatment of FH patients at very young age may finally even change the clinical phenotype of FH. It is important, therefore, to identify patients with FH early in life and start adequate treatment as soon as possible for both primary and secondary prevention. (Chapter 1)

At present, more than 700 mutations of the LDL receptor gene are known worldwide and almost 200 in the Netherlands, and this number increases every week. The finding of new LDL-receptor mutations facilitates the identification of individuals with FH, in order to initiate lipid-lowering at an early stage. (Chapter 2)

In patients with FH the severely elevated LDL-cholesterol levels cause a rapid progression of atherosclerosis as can be demonstrated by non-invasively measuring the intima media thickness (IMT) of the wall of the peripheral arteries; in FH patients the mean arterial wall IMT is twice as thick as in controls. Moreover, we have demonstrated that in FH patients with clinical manifestations of CVD the IMT is even significantly more increased compared to FH patients without CVD. (Chapter 3)
In monogenic diseases, mutations in a single gene are both necessary and sufficient to produce the clinical phenotype and to cause the disease. The impact of the gene on genetic risk for the disease is the same in all families. In complex disorders with multiple causes, variations in a number of genes encoding different proteins result in a genetic predisposition to a clinical phenotype. Pedigrees reveal no Mendelian inheritance pattern, and gene mutations are often neither sufficient nor necessary to explain the disease phenotype. Environment and life-style are major contributors to the pathogenesis of complex diseases. In a given population, epidemiological studies expose the relative impact of individual genes on the disease phenotype. However, between families the impact of these same genes might be totally different. In one family, a rare gene C (Family 3) might have a large impact on genetic predisposition to a disease. However, because of its rarity in the general population, the overall population effect of this gene would be small. Some genes that predispose individuals to disease might have minuscule effects in some families (gene D, Family 3).
IMT measurement has become a well validated non-invasive parameter for the assessment of atherosclerosis progression. We compared in two clinical trials different statin treatment modalities in a large cohort of FH patients and measured the effect of statin treatment on atherosclerosis progression by this method. (Chapter 4)

The beneficial effect of statins on cardiovascular morbidity and mortality may not only result from LDL-cholesterol reduction but also from mechanisms that modify inflammation and blood coagulation.\textsuperscript{8,9} These so called pleiotropic effects of statins have been studied extensively, but the data are not consistent. The aim of our study was to evaluate whether high dose simvastatin therapy would modulate markers of inflammation, coagulation and fibrinolysis and to evaluate whether the alterations in these markers were associated with changes in intima media thickness (IMT) of the arterial wall as marker for atherosclerosis progression in FH patients. (Chapters 5, 6 and 7)

Although FH is considered a single gene disease, there is substantial variation in the onset and severity of CVD symptoms among FH patients, which can only in part be explained by the classical risk factors such as smoking and hypertension. Even within a cohort of subjects that share an identical gene defect, there are considerable differences in the development of symptomatic disease.\textsuperscript{10} Probably additional atherogenic risk factors, of environmental and/or genetic origin, act in conjunction with the LDL receptor defect, which may explain the observed large variation in clinical manifestations in FH.

We investigated therefore, if the lipoprotein lipase mutation D9N, which is associated with reduced levels of HDL-cholesterol and elevated triglycerides, would modify the lipid and lipoprotein levels and the risk for CVD in patients with familial FH. (Chapter 8)

The distinction between single gene disorders and multiple gene disorders has become less clear. For so called single gene disorders as FH, there is one gene that may be primarily responsible for the pathogenesis, with one or more independently inherited modifier genes that also influence the phenotype.

**Background and outline of part II**

On the other hand, for a complex trait the interaction of more independently inherited pairs of alleles, most likely influenced by additional modifier genes, results in disease. Environment and life-style are major contributors to the pathogenesis of
these complex disorders. Prospective, epidemiological studies are well suited to investigate the relative impact of individual genes and environmental factors on the disease phenotype.

Atherosclerotic cardiovascular disease is therefore better understood as a multifactorial (or "complex") disease, in which not only inherited but also acquired or environmental factors play a significant role in the pathogenesis and in the complications of atherosclerosis. (Figure 1)\(^7\)

Besides the well known and already mentioned classical risk factors for CVD, one of the important mechanisms that may modulate the onset and outcome of atherosclerosis is the blood coagulation system.\(^1\)\(^-\)\(^1\)\(^1\) There are two important features that give support to this presumption. Firstly, cleavage products from the coagulation system may modulate pro- and anti-inflammatory properties of endothelial cells and leukocytes which play a key role in atherogenesis.\(^1\)\(^2\),\(^1\)\(^3\)

Secondly, platelet aggregation and clot formation as occurs in for instance myocardial infarction and ischaemic stroke, is the major complication of atherosclerosis.\(^1\)\(^4\),\(^1\)\(^5\) Subtle inter-individual differences in the response of the coagulation system caused by genetic and/or metabolic or environmental factors, may therefore modify the risk of CVD and its complications.

We performed a study on platelet aggregation and prognosis in 159 survivors of myocardial infarction participating in a secondary prevention study (the Amsterdam Metoprolol Trial) in which they were randomized for metoprolol or a placebo treatment with a follow-up of 5 years. (Chapter 1) We also studied the platelet glycoprotein IIIa Ila polymorphism, associated with platelet dysfunction, in relation to coronary artery disease progression and to the response to aspirin in participants of the Regression Growth Evaluation Statin Study (REGRESS), a placebo-controlled lipid lowering regression trial with pravastatin. (Chapter 2)

Since 1994 patients with premature atherosclerosis are referred to the outpatient 'Lipid and Premature Atherosclerosis' clinic at the AMC for CVD risk analysis and management. This gave us the unique opportunity to assess the effect of different polymorphisms and mutations on the risk of premature atherosclerotic vascular disease in this well defined patient population with CVD under the age of 50 years. (Chapter 3-6 and 8)

The genetic variations in the hemostatic system as risk factor for arterial thrombosis were reviewed from literature. (Chapter 3) One of these, the sequence variation in the 3'-untranslated region of the prothrombic (PT) gene (20210 G\(\rightarrow\)A) was recently claimed to be associated with elevated plasma prothrombin levels and an increased risk for venous and arterial thrombosis. Therefore we examined the prevalence of
the 20210 A allele in the prothrombin gene in the patients with proven premature atherosclerotic disease and in healthy controls to evaluate this mutation as a risk factor for myocardial infarction. (Chapter 4)

Very recently, mutations in the gene encoding for the ABCC6 transporter were described as the cause of Pseudoxanthoma Elasticum (PXE), an inborn disorder of connective tissue with specific skin, ocular and CVD manifestation. On several occasions we were struck by the fact that, in our patients suffering from premature cardiovascular disease, PXE was found to be concomitantly present. In the Netherlands, as in the rest of Europe, a particular premature truncation variant ABCC6 (R1141X) was quite frequent in a large cohort of PXE patients. Given the association between CVD and PXE we hypothesized that heterozygosity of this ABCC6 mutation could also confer an increased risk for CVD. We therefore studied the prevalence of the R1141X mutation in the ABCC6 gene in patients with premature CAD and in a large population based control group. (Chapter 5)

We also assessed the effect on CVD risk of three different thrombospondin mutations. The fact that thrombospondins are involved in the modulation of a wide range of processes in the vessel wall makes them plausible risk factors for premature CVD. (Chapter 6)

Epidemiological studies in which the association of iron status with atherosclerosis was assessed raised conflicting results. Therefore we tested whether genetic haemochromatosis is associated with increased atherosclerosis and we determined the prevalence of two mutations in the HFE gene related to haemochromatosis (845G → A: Cys282Tyr, and 187 C → G, His63Asp). (Chapter 7)

As mentioned before hyperhomocysteinemia is an established risk factor for atherosclerotic vascular disease. A common methylenetetrahydrofolate reductase (MTHFR) mutation is associated with hyperhomocysteinemia. We investigated the association of homocysteine levels, the MTHFR mutation and the risk for CVD in a cohort of patients with premature atherosclerosis (Chapter 8).

There are still several mutations of candidate genes under investigation in our cohort of patients with premature CVD and in a large population based control group. Such investigations are particularly important in order to reach the ultimate goal of characterising phenotypic and genetic markers for CVD. This would enable in future the establishment of individual CVD risk profiles, which might eventually result in new and individualised prognostic and preventive therapeutic measures.
References
