Familial atherosclerosis and neuroimmune guidance cues

*From in vitro assessments to clinical events*

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General Introduction and Outline of this Thesis
GENERAL INTRODUCTION

Cardiovascular disease and the lipid era

Atherosclerosis is the leading cause of cardiovascular disease (CVD) in most western developed countries [1]. Atherosclerosis is a degenerative process and the risk for atherosclerosis increases with age. However, the rate and progression of atherosclerosis accelerates if risk factors such as high low-density-lipoprotein (LDL) cholesterol plasma levels, hypertension, smoking, diabetes and obesity are present [2, 3]. These risk factors contribute to endothelial injury, which is the first step in the initiation of atherosclerosis. Monocytes and lipids in the blood can bind to the injured endothelial cells and migrate to the sub-endothelial space. Monocytes within the sub-endothelial space differentiate into macrophages which will produce pro-inflammatory cytokines. These cytokines attract even more monocytes, enhancing a inflammatory process. Macrophages and cholesterol form foam cells and eventually form an atherosclerotic plaque. This plaque will grow over the years and will rupture over time. A clot is formed causing stenosis of the artery and finally leading to lethal ischemia i.e. a myocardial infarction or stroke [4].

Since the pathophysiology of atherosclerosis is more clear, there has been an extensive search for possibilities to reduce the risk for cardiovascular disease. Big cohort studies like the Framingham Heart Study identified LDL-cholesterol as a risk factor for cardiovascular events [5]. Lowering LDL-cholesterol with 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMG-CoA reductase) inhibitors, better known as statins, reduced the CVD mortality over the years [6]. High intensity statin therapy, typically lower LDL-cholesterol levels by approximately 50% and are therefore recommended in patients at high risk for cardiovascular disease [7]. With the upcoming of PCSK9-inhibition therapy, LDL-c levels can be reduced to 0.78 mmol/L, reducing the cardiovascular risk even further [8]. However, despite all the advances in lowering LDL-cholesterol, cardiovascular disease remains the leading cause of death worldwide, with still 17.6 million deaths each year and the mortality rate is expected to rise up to 23.6 million by the year 2030 [9]. Unraveling novel non-LDL driven factors in the complex atherosclerotic process may ultimately result in new therapeutic targets to address the endemic burden of atherosclerosis.

Inflammation as residual CVD risk

In the search for non-LDL driven factors within the atherosclerotic process we started to acknowledge that the multifactorial nature of atherosclerosis is characterized by a chronic low-grade inflammation throughout all stages of atherosclerosis [10, 11]. This was based, among other things, on the finding that immune cells were found in atherosclerotic plaques [12]. The inflammation hypothesis was validated by a Mendelian Randomization study assigning inflammatory cytokine IL-6 as causal risk factor for CVD [13], and by the randomized controlled CANTOS trial, which for the first time showed a reduction in cardiovascular events in patients with known CVD upon treatment with an anti-inflammatory drug targeting IL-1β [14]. The inflammatory part of atherosclerotic plaque formation is mediated by cellular immune responses, driven by the migration of monocytes and excretion of pro-inflammatory cytokines. Within these processes, Neuroimmune Guidance Cues (NGCs) are emerging as significant regulators of monocyte migration during inflammation [15-18]. Therefore, investigating the role of NGCs in human atherosclerotic disease may identify NGCs as a novel non-LDL driven factor within the atherosclerotic process.

Neuroimmune Guidance Cues

During the development of the nervous system, axons navigate to find their target cells where they form synapses [19]. The navigation of each axon towards the proper target cell is navigated by NGCs. These NGCs involve four major families of conserved ligands an receptors, including (I) Netrins and their ‘deleted in colorectal carcinoma’ (DCC) and UNC5 receptors [20], (II) Ephrins and their Eph receptors [21], (III) Semaphorins and their Plexin and Neuropilin receptors [22], and (IV) Slits and their Roundabout (ROBO) receptors [23]. The discovery that NGC ligands and receptors are also expressed by cells outside of the central nervous system, indicated that NGCs have additional functions beyond the central nervous system. In mice studies it has been suggested that NGCs play a role in atherosclerosis as they were found to regulate the maintaining of the vascular system and they also play an important role in immune cell activation and trafficking [24-28]. Moreover, the endothelial expression of several NGCs was shown to differ between the inner (athero-resistant) aortic curve of mice, compared to the outer (athero-prone) curve of the aorta [28]. In general, Netrin-1 is possibly the best researched NGC. Netrin-1 has been shown to play an important role in atherosclerosis in mice, and ischemia-reperfusion injury by acting as a atheroprotective agent [29-32]. An important known function for Netrin-1 in the atherosclerotic field is an anti-inflammatory ability on the endothelium causing impaired adhesion and influx of monocytes and blocked macrophage efflux from the atherosclerotic plaque [29, 33]. However, human validation of these observations is lacking. Evaluating the role for Netrin-1 and other NGCs in human atherosclerosis could enhance our understanding of atherosclerosis and unravel possible new therapeutic targets to prevent or treat the most prevalent disease in the western world.

Premature Atherosclerosis

The mean age of incidence for CVD in the Netherlands is 66 years for males and 71 years for females [34]. When CVD occurs at a much younger age, defined as a cardiovascular event before the age of 50 for men and before the age of 55 for women, we call this premature atherosclerosis. Premature atherosclerosis has great medical, social and financial consequences because of the morbidity it causes at an early stage of life. A total of 6-10% of all CVD events occur at a premature age and in the
Netherlands there are approximately 40,000 hospitalizations each year, due to CVD in patients younger than 55 years [35]. Premature CVD is associated with substantially greater heritability than CVD at advanced age [36] and traditional risk factors are often absent at time of the first event [37]. With genome wide association studies (GWAS) it was possible to identify common genetic variants associated with CVD [38]. However, these variants only explained approximately 21% of the heritability of CVD, leaving a large proportion of the familial premature atherosclerosis cases unexplained [39].

Family based approach as a tool to identify new therapeutic targets.

As only 21% of the heritability of CVD can be explained by common genetic variants, studying rare genetic variants causing familial premature atherosclerosis could potentially identify new treatment targets. The advantage of this study design is that these rare genetic variants directly affect protein structure and influence the physiology of the atherosclerotic process. This way a possible direct causal relationship could be demonstrated between the genetic variant and an altered function of the protein. A very successful example of a rare genetic variant with clinical potential in families with premature atherosclerosis is the identification of a mutation in PCSK9 [40]. This gain-of function mutation in PCSK9 caused autosomal dominant hypercholesterolemia and after the initial report of the genetic association between PCSK9 and CVD in 2003, pharmaceutical companies developed a monoclonal antibody targeting PCSK9. Only 12 years later, PCSK9 inhibiting therapy was approved and proven to reduce the risk of cardiovascular events [8]. Successful examples like these pave the way for similar approaches with families suffering from unexplained premature atherosclerosis.

OUTLINE OF THIS THESIS

In this thesis we started with families who visited the outpatient clinic for families with premature atherosclerosis in the Amsterdam UMC, location Academic Medical Center. We sequenced a total of 77 NGC genes in 89 patients with extreme premature atherosclerosis and no classical risk factors for CVD. We were able to identify 200 rare variants in NGC genes. Based on high Combined Annotation Dependent Depletion (CADD) scores we identified 10 rare genetic single nucleotide variants in NGC ligands and receptors that were predicted deleterious and therefore considered to play a putative role in (premature) atherosclerosis.

Part I of this thesis focusses on the role of some specific NGCs in the development of atherosclerosis. Chapter 2 provides an overview of the function of Netrins in endothelial cell biology, including endothelial cell survival, self-renewing, barrier function and vascular tone control. In Chapter 3 and 4 we study the function of two NGC receptors, Eph Receptor B2 and Plexin A4, on atherosclerotic processes in vitro. In Chapter 5 we demonstrate a relationship between Netrin-1 and different stages of human atherosclerosis in vivo. Chapter 6 summarizes all known literature about Netrin-1 in the atherosclerotic field and its potential as a therapeutic target. After establishing a role for certain NGCs in human atherosclerosis, in Part II we try to answer specific questions in families who already suffer from premature atherosclerosis. In Chapter 7 we describe a family suffering from premature atherosclerosis where an unreported rare genetic variant in Netrin-1 is associated with cardiovascular events. The occurrence and characteristics of Brugada Syndrome in families with premature atherosclerosis is described in Chapter 8.
REFERENCES