Identification and targeting of genes in atherosclerosis

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CHAPTER 1

General introduction and outline of the thesis

Adapted from:


Chapter 1
GENERAL INTRODUCTION

The Framingham Heart study in the early ‘50’s was one of the first meticulously designed prospective studies to investigate the relative contribution of different “life style and inheritable factors (the birth of the concept of “risk factors”) for coronary artery disease (CAD). The Framingham and many following epidemiological studies have consistently shown that sex, age, LDL cholesterol, HDL cholesterol, triglyceride levels, hypertension, smoking, diabetes are the major risk factors for the development of CAD. The causality of LDL-C and hypertension in the process of CAD was established upon the notion that LDL-C lowering (statins) and blood pressure lowering drugs resulted in a dramatic decrease of CAD risk. These therapies are therefore the cornerstone in the prevention of CAD. Despite these advances, CAD remains the leading cause of death in Europe, with 46% of all deaths attributable to CAD in 2014. These data highlight the need for a better understanding of the pathophysiology of atherosclerosis and specifically addresses the need for new treatment targets.

Targets from epidemiology: HDL

Epidemiological studies have provided unequivocal evidence of an inverse association between HDL-C levels and CAD risk. In analogy to the clinical success of LDL-C lowering drugs, expectations for HDL-C increasing drugs were unlimited. This epidemiological association is supported by a number of biological plausible mechanisms. Amongst the large number of anti-atherogenic properties ascribed to HDL, its role in reverse cholesterol transport (RCT) is commonly considered to be crucial. In RCT, free cholesterol contained within macrophages in the vessel wall is taken up by the HDL particle and subsequently transported to the liver for excretion into bile. In addition to RCT, HDL has anti-inflammatory, anti-oxidant and anti-thrombotic properties. These biological activities are mainly studied in-vitro and in animal models, and translation of the epidemiological findings to a direct causal role for HDL-C in atherosclerosis has been difficult, partly due to the fact that virtually all CAD risk factors (i.e. obesity, the metabolic syndrome, smoking and physical inactivity) are associated with low HDL-C levels. The hypothesis that HDL-C directly confers biological protection against atherosclerosis has, as of yet, never been proven and, as a consequence, HDL-C has been argued to be merely a cardiovascular disease biomarker rather than an active player in atherogenesis. This notion has gained support, as therapies with an established HDL-C increasing effect were shown not to result in the anticipated decrease in cardiovascular disease risk. At the start of this thesis numerous HDL-targeted therapies were in development, which is summarized in Table 1 and reviewed elsewhere.
Chapter 1

Table 1. Overview of classes HDL-based agents tested in humans.

<table>
<thead>
<tr>
<th>Class</th>
<th>Drug</th>
<th>Route of administration</th>
<th>Stage of development</th>
</tr>
</thead>
<tbody>
<tr>
<td>ApoA-1 based</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intravenous apoA-1</td>
<td>MDCO-216</td>
<td>intravenous</td>
<td>Phase I</td>
</tr>
<tr>
<td></td>
<td>CSL-112</td>
<td>intravenous</td>
<td>Phase IIa (completed)</td>
</tr>
<tr>
<td></td>
<td>CER-001</td>
<td>intravenous</td>
<td>Phase II</td>
</tr>
<tr>
<td>Autologous HDL delipidation</td>
<td></td>
<td>intravenous</td>
<td>Phase I</td>
</tr>
<tr>
<td>apoA-1 mimetics</td>
<td>FX-5A</td>
<td>intravenous</td>
<td>Phase I</td>
</tr>
<tr>
<td></td>
<td>ETC-642</td>
<td>intravenous</td>
<td>Discontinued</td>
</tr>
<tr>
<td>apoA-1 induction</td>
<td>RVX-208</td>
<td>oral</td>
<td>Phase IIb</td>
</tr>
<tr>
<td>ABC transporter upregulation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LXR agonists</td>
<td>oral</td>
<td></td>
<td>Pre-clinical</td>
</tr>
<tr>
<td>Mir-33</td>
<td>subcutaneous</td>
<td></td>
<td>Pre-clinical</td>
</tr>
<tr>
<td>Selective PPAR modulators</td>
<td>K-877</td>
<td>oral</td>
<td>Phase II</td>
</tr>
<tr>
<td>LCAT enzyme replacement</td>
<td>ACP-501</td>
<td>Intravenous</td>
<td>Phase I (completed)</td>
</tr>
<tr>
<td>CETP inhibition</td>
<td>Anacetrapib</td>
<td>oral</td>
<td>Phase III</td>
</tr>
<tr>
<td></td>
<td>Evacetrapib</td>
<td>oral</td>
<td>Phase III</td>
</tr>
<tr>
<td></td>
<td>TA-8995</td>
<td>oral</td>
<td>Phase II</td>
</tr>
</tbody>
</table>

Targets supported by genetic studies: Triglyceride rich lipoproteins and Lipoprotein(a)

Elevated triglyceride levels are often accompanied by low HDL-cholesterol (HDL-C) levels, and both are robustly associated with CAD risk. To dissect the triglyceride and HDL-C-mediated effect on CAD risk, recent genetic studies used a Mendelian randomization strategy to assess causality. These studies suggested a direct causal association for triglyceride levels and CAD risk, independent of HDL-C levels. One of the key regulators of triglyceride metabolism is the small glycoprotein apolipoprotein C-III (apoC-III). Two independent Mendelian randomization studies have investigated the association between loss-of-function variants in the APOCIII gene, apoC-III and triglyceride plasma levels and CAD risk. One of the key regulators of triglyceride metabolism is the small glycoprotein apolipoprotein C-III (apoC-III). Two independent Mendelian randomization studies have investigated the association between loss-of-function variants in the APOCIII gene, apoC-III and triglyceride plasma levels and CAD risk. One of the key regulators of triglyceride metabolism is the small glycoprotein apolipoprotein C-III (apoC-III). Two independent Mendelian randomization studies have investigated the association between loss-of-function variants in the APOCIII gene, apoC-III and triglyceride plasma levels and CAD risk. Loss-of-function mutations (on average 46% lower apoC-III levels) were associated with a favorable lipid profile: 39–44% reduction in triglyceride levels, together with a 22–24% increase in HDL-C and a 16% reduction in LDL-C. Overall, the risk of coronary heart disease was 36–40% lower in carriers of the APOCIII mutations compared with noncarriers, highly suggestive for a causal association between apoC-III levels and CAD risk.

A completely different lipoprotein that has been the target of investigation is Lipoprotein(a) or Lp(a). Lp(a) consists of two critical elements; a central low-density lipoprotein
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(LDL)-like core containing a single molecule of apolipoprotein B100 (apoB) linked by a disulfide bridge to a signature protein called apolipoprotein(a) [apo(a)]. Initial case-control studies showed a strong association between Lp(a) and CAD risk. Mendelian randomization studies have provided evidence for a causal role of Lp(a) on CAD, building a strong case for Lp(a) as a potential therapeutic target to reduce CAD risk. For both apoC-III and Lp(a) therapeutic strategies to lower these proteins are in development, and are reviewed in detail elsewhere.

Human genetics as a tool to identify new treatment targets for atherosclerosis.

In addition to the aforementioned modifiable risk factors, CAD has a high genetic heritability, which is estimated to be about 50% based on twin studies. Technical advances have potentiated the large-scale, low-cost genotyping of common genetic variants to be studied in genome wide association studies (GWAS) in a case-control design. In the largest meta-analysis to date, 185,000 cases and controls were analyzed and these GWAS together have identified 58 common single nucleotide polymorphisms associated with CAD risk at a genome wide significance level. Together these variants only explain approximately 13% of the heritability and the mechanism linking these variants to CAD risk has not been elucidated for the majority of variants.

An alternative approach is to study rare genetic variants that directly affect protein structure and drive CAD in distinct families. Advantage of this study design is the direct relationship between genetic variant and protein function, thereby greatly increasing the possibility that the identified variant contributes to the understanding of the pathophysiology of atherosclerosis. In addition, the dramatic drop in sequencing costs in recent years has opened up the possibility of whole exome sequencing and even whole genome sequencing in key family members.

Successful examples of identification of rare genetic variants in families.

The discovery of two loss-of function mutations affecting two enzymes in the nitric oxide / cyclic GMP pathway in a large family with premature atherosclerosis illustrates the potential of this strategy. Especially as these variants were identified well before they were confirmed in GWAS studies. But, perhaps the best known example of the clinical potential of discovering rare variants in families is the identification of gain-of function mutations in PCSK9 that determine LDL-C levels, ultimately driving atherosclerosis. After the initial report of the genetic association in 2003, pharmaceutical companies subsequently developed monoclonal antibodies targeting PCSK9 and this therapy has been approved in 2016 for the treatment of Familial Hypercholesterolemia patients in the Netherlands who do not meet their target LDL-C levels. The first positive clinical outcome studies were reported in 2017, only 14 years after the initial report.
Chapter 1

OUTLINE OF THE THESIS

The starting point for this thesis are families, referred for genetic screening and cardiovascular risk assessment to our outpatient clinic. Family members are either referred based on an abnormal high frequency of CAD cases at a young age in their family or based on extreme lipoprotein levels, as a surrogate marker of increased CAD risk. We are faced with three key challenges. First to identify the underlying genetic variants and hereby identify individuals at risk in the families. Second, to understand the mechanism linking the genetic variant to the clinical phenotype and finally, to target the identified pathway and ultimately achieve CAD risk reduction.

In part I we aim to identify new genes in families with extreme HDL and or TG levels. In Chapter 2 and 3 we study two families with very low HDL-C levels. In these families we study rare variants in LRP1 and ABCA8 and report the effects of these variants on HDL metabolism. In Chapter 4 we use the same approach, only with a focus on triglyceride levels and study the effects of two new APOC3 mutations on triglyceride metabolism.

The most promising therapeutic targets in HDL, triglyceride and Lp(a) metabolism are studied in part II. In Chapter 5 and 6 we investigate the relationship between plasma apoC-III levels, lipoprotein-associated apoC-III levels and CAD risk and other lipoproteins in a large prospective study of 2,711 individuals of whom 832 developed CAD during follow-up. In Chapter 7 and 8 we focus on HDL. First, we study the difference between HDL-C and apoA-I as predictors of CAD risk in three large prospective cohorts. Second, we study the effects of the CETP-inhibitor TA-8995 on cholesterol efflux capacity, a measure of HDL-functionality. Finally, in Chapter 9, we test the effect of apo(a) antisense therapy, a potent new Lp(a) lowering agent on the effect of Lp(a) levels and to assess whether reversal of a pro-inflammatory monocyte phenotype is possible.

The focus of part III is to identify rare variants in large pedigrees with an observed autosomal dominant pattern of premature atherosclerosis, defined as a first CAD event <50 years in men and <55 years in women. Key aspect of our approach is the selection of a very stringent phenotype, where we only selected families with a minimal number of traditional CAD risk factors, like LDL-C, BMI and smoking, to increase the likelihood to identify new variants contributing to the development of atherosclerosis. In Chapter 10, 11 and 12, we describe three families with premature atherosclerosis that are associated with previously unreported rare genetic variants in KERA, MCF2L and SUSD2.
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17. Kamstrup PR et al. Genetically elevated lipoprotein(a) and increased risk of myocardial infarction. JAMA 2009;301(22):2331–9.