Genetic and biochemical riks factors in coronary artery disease
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Summary and conclusion

This thesis explores from several different angles the pathogenesis of atherosclerosis and its principal clinical manifestation coronary artery disease. First, the thesis investigates three different fields of research essential in atherogenesis: (1) lipids, lipoproteins and apolipoproteins, (2) oxidation and inflammation, and (3) coagulation and fibrinolysis. Second, within each field, research focuses on the triangulation between genes, proteins, and cardiovascular risk. In some chapters, we explore the concept of Mendelian Randomization as a method to mutually reinforce the value of classical and genetic epidemiology. In several other chapters, we study the relationships among protein levels and cardiovascular risk, genetics and cardiovascular risk, or the interaction between protein levels and genetics on cardiovascular risk. The implications of the findings for our understanding of atherosclerosis and the prevention of coronary artery disease are discussed.

Part I: Lipids, lipoproteins and apolipoproteins
Chapter 2 is a meta-analysis of genetic association studies that assessed the relationships between three different variants in the apolipoprotein B (apoB) gene, lipid levels and coronary artery disease risk. We observed that homozygotes for the XbaI X+ allele had significantly elevated levels of LDL cholesterol (LDL-c) and apoB but a decreased risk of coronary artery disease. Homozygosity for the signal peptide deletion allele was associated with similarly increased levels of LDL-c and apoB, and with an increased cardiovascular risk. Subjects homozygous for the rare EcoRI allele had significantly decreased levels of total and LDL cholesterol, but their risk of coronary artery disease was not significantly altered. These data suggest that besides the direct relationships among apoB levels, hypercholesterolemia and cardiovascular risk, other factors such as molecular variants of the apoB molecule may have an effect on cardiovascular risk as well. In chapter 3 we compared the value of lipids with that of apolipoproteins in the prediction of cardiovascular risk. Specifically, we performed a prospective nested case-control study nested in the EPIC-Norfolk cohort and quantified the levels of low density lipoprotein cholesterol (LDL-c), high density lipoprotein cholesterol (HDL-c), apolipoprotein A-I (apoA-I), and apoB. We observed that compared to LDL-c and HDL-c, the gold standards in dyslipidemia management and the recommended parameters for risk prediction, serum levels of apoA-I and apoB were substantially better predictors of the risk of future coronary artery disease among apparently healthy men and women. Among all parameters tested, the ratio apoB/A-I was the strongest predictor of cardiovascular risk even after adjustment for LDL-c and HDL-c. Our observations provide a strong argument for the use of apolipoproteins in cardiovascular risk prediction.
Chapter 4 describes the relationships among variants in the gene for cholesteryl ester transfer protein (CETP), lipid parameters and the risk of cardiovascular disease. The clinical relevance lies in the fact that pharmaceutical companies have developed inhibitors of CETP activity which have been shown to raise HDL-c levels. Research on naturally occurring variants in the CETP gene has yielded numerous insights that have been relevant for understanding the lipoprotein metabolism, and crucial to the development of pharmacological CETP inhibition. We conclude that the currently available evidence suggests that several genetic variants in the CETP gene are associated with altered CETP plasma levels and activity, HDL-c plasma levels, LDL and HDL particle size, and possibly the risk of cardiovascular disease. In chapter 5, we performed a large-scale analysis to assess the relationships among the TaqIB variant in the CETP gene, HDL-c levels and cardiovascular risk. We performed a meta-analysis of individual patient data derived from 7 large population-based studies (each >500 individuals) and 3 randomized placebo-controlled pravastatin trials. After adjustment for relevant cardiovascular risk factors, B2B2 individuals had 0.11 mmol/l higher HDL-c levels than B1B1 individuals. Consistently, the B2 allele was associated with a significantly lower risk of CAD. This relationship disappeared upon adjustment for HDL-c levels. We concluded that the CETP TaqIB variant is firmly associated with HDL-c plasma levels, and as a result with the risk of CAD. Chapter 6 investigates the relationship between CETP, HDL-c levels, and cardiovascular risk, but this time we assessed CETP at the protein level. Because evidence exists that the effect of CETP depends on metabolic context, in particular on triglyceride levels, we also tested for effect modification by triglyceride levels. A nested case-control study was performed in the prospective EPIC-Norfolk cohort study. CETP levels were not significantly different between cases and controls. CETP levels were significantly related with plasma levels of total cholesterol, LDL-c, and HDL-c. The risk of CAD increased with increasing CETP quintiles, such that subjects in the highest quintile had an adjusted odds ratio of 1.43 versus those in the lowest. Among individuals with triglyceride levels below the median, no relationship between CETP levels and CAD risk was observed, but this relationship was strong among those with high triglyceride levels, such that those in the highest CETP quintile had an odds ratio of 1.87. We concluded that elevated CETP levels are associated with an increased risk of future CAD in apparently healthy individuals, but only in those with high triglyceride levels. CETP levels have an inverse relationship with HDL-c levels. In addition, a strong and inverse relationship exists between HDL-c levels and cardiovascular risk. Pharmaceutical companies have jumped to the development of inhibitors of CETP activity under the assumption that CETP inhibition would result in raised HDL-c levels and a concomitant reduction in cardiovascular risk. Chapter 7 reviews the currently available evidence on these inhibitors of CETP activity. First, we discuss the evidence from epidemiological studies showing that the relationship between low CETP levels and high HDL-
c levels can indeed be extrapolated to a reduced risk of cardiovascular disease. Second, we summarize the data available on the safety and efficacy of CETP inhibitors, and we speculate on their potential future role in the treatment of dyslipidemia and the prevention of cardiovascular disease.

Part II: Oxidation and Inflammation
Lipoproteins are atherogenic because they penetrate the arterial wall, undergo various modifications, and initiate a self-perpetuating inflammatory process that ultimately leads to atherosclerotic plaque formation. One of the proteins that modifies lipoproteins in the vessel wall, is type II secretory phospholipase A2 (sPLA2). Its activity yields LDL particles that interact more readily with proteoglycans in the arterial wall, thereby increasing their residence time in the vessel wall, and as a consequence, their chance to undergo modification. In addition, sPLA2-mediated hydrolysis of phospholipids yields among others, lysophospholipids and free fatty acids such as arachidonic acid, known precursors of various proinflammatory mediators such as leukotrienes and prostaglandins. Finally, sPLA2-modified LDL particles are more susceptible to oxidation. In chapter 8, we assessed the relationship between serum levels of sPLA2 and the risk of future coronary artery disease among apparently healthy men and women. We observed that people in the highest sPLA2 quartile were at an increased risk of developing coronary artery disease, even after adjustment for traditional cardiovascular risk factors and CRP. After undergoing various molecular modifications, lipoproteins in the arterial wall may initiate the inflammatory system because several molecular patterns of modified lipoproteins show molecular mimicry with infectious pathogens. For instance, the innate inflammatory system uses toll-like receptor-4 to recognize phosphatidyl choline (PC), which characterizes the cell membrane of gram-negative bacteria, including S. pneumoniae. Modified lipoproteins may expose these PC groups, thereby triggering an inflammatory response. In chapter 9, we investigated whether variants of the toll-like receptor-4, which are known to blunt the inflammatory response lipopolysaccharide inhalation, affected the risk of cardiovascular events. We observed that among people with prevalent CAD, carriers of these variants have an increased risk of cardiovascular events. Pravastatin therapy reversed this relationship. Subsequently, the triggered inflammatory response facilitates recruitment of peripheral blood monocytes into the arterial wall. The vascular wall itself orchestrates this process by modulating the expression of a wide variety of cytokines that attract leukocytes, enable rolling leukocytes to adhere to the endothelium, and facilitate trans-endothelial immigration into the subendothelial space. Interleukin-8 is one of these cytokines expressed by endothelial cells. Chapter 10 describes the observation that apparently healthy people with high interleukin-8 levels have an increased risk of future cardiovascular events. One of the other inflammatory proteins orchestrating the inflammatory process that facilitates atherosclerosis, may be macrophage
migration inhibitory factor (MIF). It may play an important role in facilitating the adhesion and subsequent entry of monocytes into the arterial wall as well. In chapter 11, we found that plasma levels of MIF were associated with an increased risk of future cardiovascular events among apparently healthy men and women. This inflammatory aspect of atherosclerosis has started to gain attention in recent years. In particular, the use of C-reactive protein as an inflammatory marker in clinical practice has been a topical debate. CRP levels undoubtedly predict future cardiovascular events, but opponents of its use in clinical practice have argued that its added value is only marginal. In chapter 12, we investigated the added value of C-reactive protein levels on top of traditional cardiovascular risk factors in predicting future cardiovascular events in a contemporary Western population.

**Part III: Coagulation and fibrinolysis**

Clinical manifestations of atherosclerosis occur due to arterial occlusion. The principal cause is occlusive thrombosis after atherosclerotic plaque rupture or erosion. Thrombus formation results from the interaction between activated platelets and the coagulation system. In particular, activated platelets which expose numerous IIb/IIIa receptors, bind several fibrinogen fibers thereby causing the formation of a thrombus network. A genetic variant of glycoprotein IIIa, one of the components of the IIb/IIIa receptors, has been associated with an enhanced adhesive phenotype, but observations have been inconsistent. In chapter 13, we studied whether this inconsistency may be explained by environmental factors. We observed that the variant allele was indeed associated with an increased risk of cardiovascular events, but only among people with high fibrinogen levels. Besides this variant of glycoprotein IIIa, several other genetic variants of haemostatic proteins have been implicated in determining the risk of cardiovascular events as well. However, as often in genetic association studies, the results have been inconsistent. An important factor in this inconsistency may be lack of statistical power. In chapter 14, we therefore performed meta-analyses on all studies that associated the presence of four genetic variants with the risk of myocardial infarction. The factor V Leiden and prothrombin G20201A mutations did not significantly correlate with myocardial infarction. Homozygosity for the fibrinogen-455A allele was significantly associated with a decreased risk of myocardial infarction, whereas the PAI-1 4G4G genotype was significantly associated with increased risk. As described in chapter 13, environmental factors may determine the relationship between genetic variants and outcome. The molecular interaction between factor XIII and fibrin fibers play a role in the later phases of thrombus formation. A recent in vitro study suggested that the factor XIII Val34Leu variant may increase the density of thrombus structure, thereby enhancing its risk profile. In chapter 15, we tested whether the in vitro finding could be reproduced in a prospective study among apparently healthy men and women. We observed no statistically
significant interaction between fibrinogen plasma levels and the factor XIII Val34Leu variant in determining the risk of cardiovascular events. However, our study may have lacked statistical power to detect such an interaction.