Chapter 1

General introduction and outline of the thesis
PART 1

Restenosis, stent malapposition and other aspects of vascular disease.

After a percutaneous coronary intervention (PCI) with stent placement re-endothelialization should occur as part of a normal wound healing response. In a considerable amount of patients treated with a bare metal stent (BMS) a dysregulation of this response leads to neointimal hyperplasia and partial reocclusion of the intervention site, which is known as restenosis. As nowadays the use of stents, either bare metal or drug-eluting, has become standard, which largely rules out restenosis due to vascular recoil, we will focus on in-stent restenosis, which is mainly due to neointimal proliferation. After bare metal stenting, the incidence of target vessel revascularization, which is considered to be the most important endpoint by regulatory agencies, is approximately 10%. From the combined data of randomized controlled trials we know that patients treated with a drug eluting stent (DES) have approximately half this risk. However, disadvantages related to late acquired stent malapposition and delayed endothelialization with longer required use of P2Y12 antagonists, do not favour the use of DES in every patient. Taking into account that DES have not eradicated restenosis completely and that at least certain groups of patients benefit more from BMS, it is important to improve risk stratification and to tailor individual therapy. However, only few clinical and lesion-related risk factors have been found to predict the development of restenosis. From many studies, with different indications for PCI and different endpoint definitions, we have learned that Diabetes Mellitus is the only strong consistent clinical predictor of restenosis. Hypertension has also been reported to increase the risk for restenosis. In addition, several lesion-related and procedural characteristics such as stenosis severity (before stenting) and residual stenosis (after stenting), which were regularly reported to be associated with restenosis risk, can be used as clues to select the appropriate treatment.

Clinical risk factors such as diabetes and hypertension are also long known to play a role in the development of atherosclerosis, a disease process with several similarities to restenosis, leading to world-wide frequently occurring diseases such as angina pectoris, myocardial infarction and stroke. Restenosis, coronary atherosclerosis and also atherosclerosis in other arteries are proliferative processes driven by inflammation. However, the relative importance of risk factors differs between these diseases. In contrast to restenosis, the development of atherosclerosis is strongly influenced by circulating lipids and smoking. And the precise risk profile in atherosclerosis even differs depending on the location of the plaque. Stroke is relatively more determined by hypertension, whereas plasma cholesterol is more important in coronary disease.
The importance of genetics

The different aspects of vascular disease have in common that multiple genetic factors play an important role in their development. The actual impact of any clinical risk factor on an individual depends for a large part on his/her genetic susceptibility to this factor. The value of genetics in cardiovascular disease is corroborated by the strong predictive value of a positive family history and further confirmed by twin studies showing that death from coronary artery disease at an early age of one twin is a strong predictor of the risk in the other twin.\textsuperscript{10} Many genes in inflammatory and proliferative processes, but also in processes important in hemostasis, cell signaling, lipid metabolism and endothelial function, have already been found to play a role in vascular disease.\textsuperscript{11, 12}

The first chapter of part 1 of this thesis (chapter 2) will review current views on the role of genetics in restenosis after BMS placement and acquired malapposition after treatment with a DES. The remainder of part 1 (chapter 3-7) will discuss new data further establishing the important influence of genetic factors in the development of adverse events after PCI and other aspects of vascular disease such as stroke. Each chapter addresses a specific process and its relative importance in one of these vascular diseases, mostly restenosis after PCI in the GENDER-study, which included 3104 patients after successful PCI for stable angina pectoris or non-ST-elevation myocardial infarction.

\begin{table}[h]
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\begin{tabular}{|l|l|l|l|}
\hline
Part 1 & Process & Endpoint & Population \\
\hline
Chapter 3 & Hemostasis & Restenosis & GENDER \\
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Chapter 4 & VSMC prol./hemostasis & Restenosis/stroke & GENDER/PROSPER \\
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Chapter 5 & Matrix formation & Restenosis & GENDER \\
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Chapter 6 & VSMC function & Restenosis & GENDER \\
\hline
Chapter 7 & Endothelial function & Restenosis & GENDER \\
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Chapter 8 & Inflammation & Stent malapposition & MISSION \\
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Considering that a reliable risk estimate cannot be made on the basis of clinical factors, genetic epidemiology can provide new risk markers to improve risk stratification. It can also lead to new insights in the pathophysiology and thereby provide new targets for therapy.

PART 2

Epigenetics

Since long we know that the final phenotype of an organism, and also its tendency to develop disease, is the result of the interplay between nature and nurture. It has now become clear that environmental influence (nurture) can exert its effect not only by
influencing the code of DNA, but also (far more easily) by regulating gene expression without changing the code.\textsuperscript{13, 14}

Early research by Kaati et al., investigating early nutritional influences on cardiovascular mortality, already demonstrated heritability of environmental effects.\textsuperscript{15} They exploited records of annual harvests from an isolated community in northern Sweden that go back as far as 1799 to explore the effects of food availability across three generations. Kaati and coworkers showed a remarkable effect of food availability during the slow growth period (SGP) just before puberty of the paternal grandfather on the longevity of the probands. Scarcity of food in grandfather’s SGP was associated with a significantly extended survival of his grandchildren for many years, whilst food abundance was associated with a greatly shortened life span of the grandchildren.\textsuperscript{15} These findings are most probably an example of non-DNA sequence-related heredity, which we now refer to as “epigenetics”. In contrast to classical mendelian views on inheritance, epigenetics focuses on the heredity of environmental effects, a phenomenon that is called ‘epigenetic inheritance’. Although the precise mechanism remains unknown, it seems likely that the phenomenon observed by Kaati et al. is the result of DNA-methylation, the best understood example of epigenetic modification which is known to be involved in ‘genetic imprinting’.\textsuperscript{16} Methylation of DNA leads to silencing of genes and is maintained during cell division by virtue of the enzyme DNA methyltransferase I.

Several other findings implicate genetic imprinting in similar transgenerational effects. Mice experiments with the Agouti-allele, which normally leads to a yellow phenotype, have shown that a methyl-rich diet, when given to pregnant mice not carrying the Agouti-allele, could silence the Agouti-gene in their offspring.\textsuperscript{17} Especially interesting was the finding that methylation of the Agouti-allele was more likely to be maintained when the allele was maternally inherited. In humans, investigations into the underlying cause of the Prader-Willi and Angelman syndromes have lead to the discovery of a similar example of epigenetic inheritance. Due to a different DNA-methylation imprint, loss of the paternal copy of 15q11-q13 was found to lead to Prader-Willi syndrome, which is characterized by obesity, a short stature, extreme flexibility and delayed puberty, whereas maternal deletion of the same region on chromosome 15 has been shown to lead to Angelman syndrome,\textsuperscript{18} a neuro-genetic disorder characterized by intellectual and developmental delay, sleep disturbance, seizures, jerky movements (especially hand-flapping) and frequent laughter or smiling.

A second well-studied example of epigenetic change is chromatin modification; rearrangement of nucleosomes, which include covalent post-translational modifications of histone tails. Of several types of chemical modification, also including methylation, phosphorylation and ubiquitinylation, especially acetylation of lysine residues in the histone tails is considered a key process in gene regulation and is the main subject of part
II of this thesis. The histone acetylation status is regulated by two sets of enzymes: lysine acetyltransferases (KATs) and lysine deacetylases (KDACs). KATs acetylate histones by transfer of an acetyl-group to the ε-portion of lysine residues, which results in an open modification of chromatin structure and in accessibility of DNA to transcription factors and recruitment of the basal transcription initiation machinery.\textsuperscript{19, 20} Conversely, gene repression is mediated via KDACs, which remove acetyl groups and counteract the activity of KATs resulting in a closed chromatin structure. Unlike DNA methylation, a possible mechanism of maintaining histone acetylation through generations is not well understood. However, the modern definition of epigenetics does not require meiotic heritability, but should mention DNA modifications, other than DNA sequence variation, that carry information content during cell division.\textsuperscript{14} Although a replicating enzyme has yet to be discovered, histone acetylation changes might turn out to be self-perpetuating.\textsuperscript{21} A possible mechanism is suggested by the phenomenon of ‘spreading’ of silencing in yeast which is mediated by the histone deacetylase activity of Sir2p.\textsuperscript{22} Sir2p-induced hypoacetylation of nucleosomes attracts other Sir proteins and leads to spreading of silent chromatin along the chromosome in \textit{S. cerevisiae}. Irrespective of these findings the process of histone acetylation/deacetylation is generally accepted as one of the pillars in epigenetic research.

Epigenetic regulation of gene expression is also known to be important for cell differentiation. In every cell two thirds of our more than 25.000 genes are repressed by epigenetic mechanisms and every cell-type expresses a totally different set of genes. Furthermore, epigenetic mechanisms have been found to play a role in the development of human complex diseases such as cancer.\textsuperscript{23, 24} Chapter 9 of Part 2 of this thesis will discuss new insights in the role of epigenetic gene regulation (chromatin remodeling) also in determining susceptibility to cardiovascular disease, a new area of research. This chapter will also discuss the reversibility of epigenetic changes and the promising role of these mechanisms in the development of future therapy.

**Epigenetic epidemiology**

Thus far, the main focus has been to investigate the environmental influence on epigenetic processes. From literature we know that epigenetic differences arise during the lifetime of monozygotic twins\textsuperscript{25} and that oxidative stress influences the balance between KATs and KDACs in favour of KATs, leading to an increase in inflammation.\textsuperscript{26} Part 2 of this thesis introduces the concept that epigenetic processes are also under genetic control and that, besides the environment, genetic variation in genes encoding KATs and KDACs could also be an important determinant of susceptibility to complex human diseases such as cardiovascular disease.

It has already been shown that single gene disorders of the epigenetic machinery also impair normal gene expression. Lack of the MeCP2 protein, which recognizes
methylated DNA and helps to repress gene expression, is known to lead to the Rett syndrome. Similarly, loss of one functional copy of the CREB-binding protein (CBP), a transcriptional co-activator with intrinsic KAT-activity, underlies all abnormalities in patients with the Rubinstein-Taybi Syndrome. Single nucleotide polymorphisms in the sequence of these epigenetic genes could act generally on disease susceptibility by affecting the fidelity of the histone acetylation machinery. In the worm C. Elegans, the genetic variants which were found to have the broadest influence on gene expression, affecting many signaling pathways, were found to be present in chromatin-modifying genes. Furthermore, in humans, recent finding demonstrate that common genetic variants in the CBP gene are associated with altered cognitive function in the PROSPER-study, which included 5804 elderly patients at risk for vascular disease.

Chapter 10 of this thesis will focus on this relatively new area of research, which we call 'epigenetic epidemiology'. In this chapter the PCAF gene will be introduced, encoding a co-activator with intrinsic KAT-activity and a broad influence on inflammatory and proliferative gene expression. This chapter addresses its newly identified role in cardiovascular disease and the significance of common genetic variation in epigenetic genes in determining coronary heart disease mortality.
REFERENCES


