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Genetic, clinical and experimental aspects of restenosis : a biomedical perspective

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SUMMARY AND CONCLUSIONS

This thesis examines different clinical factors in relation to restenosis. However, it has its main focus on different polymorphisms from numerous genes in relation to restenosis after Percutaneous coronary interventions (PCI). Restenosis is a multifactorial process, therefore only a limited part of the number of candidate genes that are potentially involved in restenosis can be described. For that reason our study has its main focus on inflammatory markers. The inflammatory reaction is known to be highly important in the process of restenosis. To examine various candidate genes and their polymorphisms we made use of the GENetic DEterminants of Restenosis (GENDER) study, which is a multicenter follow-up study. It was designed to study the association between various gene polymorphisms and clinical restenosis, defined by Target Vessel Revascularization (TVR). Patients were eligible for inclusion if they were successfully treated for stable angina, non-ST-elevation acute coronary syndromes or silent ischemia by PCI. Patients treated for acute ST elevation myocardial infarction (MI) were excluded. In total, 3,104 consecutive patients were included in this prospective multicenter follow-up study. In the different chapters we describe the study population and the clinical factors and genes with their polymorphisms investigated. Furthermore, we made use of a mouse model to further improve our understanding of the development of restenosis and we have investigated for a number of polymorphisms their functional effect on protein level. The implications of our findings for the understanding and possible prevention of restenosis are discussed.

PCI is a frequent therapeutic option in patients with Coronary artery disease (CAD). However, clinical restenosis remains a significant problem. Identifying patients at increased risk for restenosis may improve stratification of patients to individually tailored treatment. A number of clinical characteristics have been shown to be associated with an elevated risk of restenosis after PCI. Thus far, however, patients cannot be stratified with regard to risk for coronary restenosis based only upon clinical or procedural risk factors. There is evidence that genetic factors explain part of the excessive risk for restenosis independently of conventional clinical variables after PCI. **Chapter 2** is a review that describes the different genes and their polymorphisms associated with restenosis at the time we started our study. This review shows that genes indeed can help to improve the stratification of patients at increased risk for restenosis. However, many of the published studies were of relatively small sample size, which sometimes show more positive outcomes than the larger studies, this is possibly due to publication bias towards more positive results. The small sample size studies also exhibit wide confidence intervals. Furthermore, it showed that still much research has to be done.

Chapter 3 describes the GENDER population, which is the population we used to perform our DNA analysis. This chapter shows that follow-up (9.6 months, IQR 3.9) was complete in 3,146 (99.3%) patients with a mean age of 62.1±10.7 years. Of them 896 (28.5%) were female, 459 (14.6%) had diabetes and 1,459 (46.4%) had multivessel disease. Most patients (2,105, 66.9%) were treated for stable angina. Of all patients 819 (26.0%) were treated for multiple lesions, 2,340 (74.4%) underwent stenting and 820 (26.1%) received glycoprotein IIb/IIIa inhibitors. All stented patients received life-long aspirin and ticlopidin/clopidogrel during at least 1 month after the procedure. Target vessel revascularization, which was our primary endpoint, during follow-up by either CABG or PCI was necessary in 304 patients (9.7%). Thirty-three (1.1%) patients died of cardiac disease and 22 (0.7%) patients suffered from MI attributable to the originally treated vessel. Overall a need for revascularisation, cardiac death or MI occurred in 346 patients (11.0%), at 9 and 12 months these event-rates were 10.2 and 12.0%, respectively. When we restricted the endpoint to TVR, diabetes, hypertension, residual stenosis more than 20% of the luminal diameter, treatment of total occlusions were associated with an increased risk of TVR. As expected, successful stent placement was associated with a lower TVR rate. Out of 3,146 patients 42 had an event in the first 30 days. These patients were excluded in the gene analysis, since these events were attributable to sub-acute stent thrombosis or occluding dissections and not likely to restenosis.

Diabetes has already been shown to be an independent predictor of restenosis after PCI. However, only limited data are available on the effect of metabolic syndrome on restenosis in patients undergoing PCI. To assess the role of metabolic syndrome in the development of restenosis, we performed an analysis in a subpopulation of patients from the GENDER study. This subpopulation of GENDER consisted of 901 patients, 448 (49.7%) of whom had metabolic syndrome. This study as described in **chapter 4**, demonstrates that metabolic syndrome is not associated with TVR or the combined end point after PCI. Furthermore, accumulating characteristics of metabolic syndrome were neither associated with increased risk of TVR nor with the combined end point. Therefore, PCI has equal beneficial results in patients with or without metabolic syndrome. This is important information in light of the pandemic proportion of metabolic syndrome facing the medical community.

To scan a broad spectrum of genes and their polymorphisms and thereby get an overview of which processes are involved in the development of restenosis, we started our gene study by performing two large array analyses. In **chapter 5** one

of these array analysis, which consists of forty-eight polymorphisms in 34 genes in pathways possibly involved in the inflammatory process were analysed. The 16Gly variant of the beta-2 adrenergic receptor gave an increased risk of TVR. The rare alleles of the CD14 gene (-260T/T), colony stimulating factor 2 gene (117Thr/Thr) and eotaxin gene (-1328A/A) were associated with decreased risk of TVR. However, using multiple testing corrections by means of permutation analysis the probability to find four significant markers by chance was 12%.

Although increased fibrinogen levels have been shown to be associated with increased risk of CAD, the effect of preprocedural fibrinogen levels on in-stent restenosis is largely unknown. Fibrinogen is involved in coagulation and inflammation, both important processes in restenosis. Moreover, the -455 G/A polymorphism of the fibrinogen β -gene is associated with baseline plasma level or acute phase increase of fibrinogen. To examine the role of this polymorphism in the development of restenosis, we made use of a subpopulation of the GENDER study. This subpopulation consisted of 2,309 patients, all of whom received a stent. As described in **chapter 6**, the presence of the -455G/A polymorphism in the fibrinogen β -gene and preprocedural fibrinogen level is not associated with an increased risk of TVR or combined endpoint in a patient population with coronary stent placement. Therefore, we showed that these parameters are not important in the stratification of patients at risk for restenosis pre-stenting.

Variations in the lipoprotein lipase (LPL)-gene have been implicated in a number of pathophysiological conditions associated with CAD. In **chapter 7** we examined the impact of polymorphisms in the LPL-gene on restenosis. These patients were genotyped for four different LPL-gene polymorphisms. Using multivariable analysis, carriers of the 447Ter allele of the LPL-enzyme showed a lower risk of TVR compared to 447Ser homozygotes. The LPL C/G polymorphism (Ser447Ter) resulting, in a truncation of the two C-terminal amino acids of the mature LPL-protein, appears to be an important protective factor for TVR in man. LPL's role in this process was further established in a mouse model, where LPL-expression was very strongly upregulated in the target arterial wall, suggesting a contribution of this lipolytic enzyme to restenosis. Possibly, LPL Ser447Ter genotyping may lead to better risk stratification and tailored therapy in the prevention of restenosis after PCI.

TNF α , a key regulator of inflammatory responses, may exert critical influence on the development of restenosis after PCI. **Chapter 8** describes the systematic genotyping for six polymorphisms in the TNF α gene. The role of TNF α

in restenosis was also assessed in ApoE*3-Leiden mice, TNF α knockout mice and by local delivery of a TNF α biosynthesis inhibitor, thalidomide. The -238G-1031T haplotype of the TNF α gene increased clinical and angiographic risk of restenosis ($P=0.02$ and $P=0.002$, respectively). In the mouse model of reactive stenosis, arterial TNF α mRNA was significantly, time-dependently, upregulated. Mice lacking TNF α or locally treated with thalidomide showed a reduction in reactive stenosis ($P=0.01$ and $P=0.005$, respectively). Therefore, TNF α genotype may be used as a risk marker for restenosis and may contribute to individual patient screening prior to PCI in clinical practice. Inhibition of TNF α may be an anti-restenotic target strategy.

Caspase-1 (also known as interleukin-1 β converting enzyme/ ICE), Interleukin-1 receptor-1 (IL-1r) and protein tyrosine phosphatase non-receptor type 22-gene (PTPN22) are important mediators in the inflammatory response. As a result **chapter 9** describes whether polymorphisms in the genetic code of these three candidate genes are related to the risk of development of restenosis after PCI. Genotyping was performed by MassArray platform (Sequenom) for the 5352G/A (L235L) polymorphism in the caspase-1 gene and the IL-1r 7464C/G (A124G) polymorphism and by Taqman analysis for the 1858C/T (R620W) polymorphism of the PTPN22-gene. After correcting for clinical variables the caspase-1 polymorphism was significantly associated with TVR (RR; 2.2, 95% CI; 1.32-3.76). The other two polymorphisms did not show a significant association. Caspase-1 is associated with the maturation of the pro-inflammatory cytokines IL-1 β and IL-18 and with apoptosis. To examine the functional effect of the caspase-1 polymorphism, plasma IL-1 β -levels were measured by enzyme-linked immunosorbent assay in lipopolysaccharide (LPS) stimulated whole blood from a sub population of patients. This analysis demonstrated an increase in IL-1 β levels for the 5352AA genotype and although not statistically significant these results corroborated our hypothesis that having the 5352AA genotype increases the risk of developing restenosis.

Finally, the last chapter, **chapter 10**, examines four different polymorphisms in the IL-10 gene. Interleukin (IL)-10 is an important immunosuppressor cytokine, involved in the regulation of many aspects of immune responses. Genotyping was performed by MassArray platform (Sequenom) for the following polymorphisms in the IL-10 gene: -592C/A, -2849G/A, -1082G/A and +4259A/G. After adjusting for clinical variables three out of the four polymorphisms were still significantly associated with TVR (-2849G/A: RR; 1.7, 95%CI; 1.2-2.5, -1082G/A: RR; 1.4, 95%CI; 1.1-1.8, and +4259A/G: RR; 2.0, 95%CI; 1.4-2.8). The -592C/A polymorphism (RR of 0.9, 95%CI; 0.8-1.1) did not show a significant association.

In conclusion the results of this study showed that three polymorphisms of the IL-10 gene are associated with the development of restenosis after PCI. Therefore, IL-10 is a risk marker for the development of restenosis and may provide indications for improving of individual treatment, since it may be a new target point for drug-eluting stents.

Conclusions

Restenosis is the main drawback of percutaneous coronary interventions (PCI). Genetic variance poses an opportunity to enhance stratification of individuals who will be more prone to develop restenosis. Furthermore, it can contribute to optimization of medication and proper assignment of revascularization strategies. However, association studies have some difficulties that can be limited by following certain rules. For instance, population sizes should be large enough and results need confirmation. In the GENDER study we tried to incorporate these rules. This is a large prospective follow-up study. We performed different techniques to examine various genes and their polymorphisms, mostly involved in the inflammatory process. From our analysis we demonstrated an increased risk in developing restenosis with the following polymorphisms: the 16Gly/Gly genotype of the beta-2 adrenergic receptor, 5352AA genotype of the caspase-1 gene, the -2849AA, the -1082AA and the +4259GG genotypes of the IL-10 gene. The rare alleles of the CD14 gene (-260T/T), colony stimulating factor 2 gene (117Thr/Thr) and eotaxin gene (-1328A/A) were associated with decreased risk of TVR. Moreover, carriers of the -238GA/AA and -1031TC/CC genotype of the TNF α gene demonstrated a lower risk for developing restenosis. The LPL C/G polymorphism (Ser447Ter) resulting, in a truncation of the two C-terminal amino acids of the mature LPL-protein, also appears to be an important protective factor for TVR in man. Patients having the -455G/A polymorphism in the fibrinogen β -gene showed no association with TVR. Our results have contributed to a better understanding of the restenotic process. From this study possibly new drug targets for the drug-eluting stents can be developed. The clinical and practical introduction of gene polymorphisms as novel risk factors will need further research to confirm our results. Furthermore, it will depend on the availability of rapid and affordable genotyping techniques. Since restenosis is a multifactorial disease more candidate genes will be examined in the GENDER study with the main focus on epigenetic factors.