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

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GENETIC PREDICTIVE FACTORS IN RESTENOSIS

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Abstract

Restenosis is still the main drawback of percutaneous transluminal coronary angioplasty (PTCA). It is thought to be a multifactor process where recoil of the vessel, neointimal proliferation and thrombus formation are thought to play a role. Until now it has proven difficult to predict restenosis on clinical and procedural grounds, however genetic epidemiology might provide more insights. In this review several genetic variables, i.e. polymorphisms that were determined in relation to restenosis are described. The single nucleotide polymorphisms (SNP's) described in the literature so far involve; the renin-angiotensin system, platelet aggregation, the inflammatory response, matrix metalloproteinases, smooth muscle cell proliferation, lipids and oxidative stress and nitric oxide. Nowadays DNA-micro arrays have been developed which make it possible to test 50 or 60 polymorphisms at once. However the risk of error due to multiple testing should be kept in mind.

The results of the studies described should be interpreted with care. Many of the published studies are 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. On the other hand, one must take into account that the process of restenosis is a multifactorial one and it is likely that multiple genes are involved. Thus, relatively small odds ratios relating to single gene contribution to restenosis can be of paramount importance when encompassed in the overall picture. Although still much research has to be done, stratification according to genetic make-up may enable tailoring of the interventional treatment to the individual patient.

Introduction

Over the past twenty years, percutaneous transluminal coronary angioplasty (PTCA) has gained wide acceptance as the procedure of choice in many patients with atherosclerotic coronary artery disease. However, a major drawback of PTCA still is restenosis of the treated vessel. This occurs in 12-60% of the patients within 6 months after intervention, depending on patients' characteristics and the interventional techniques used.^(1;2)

The restenotic process is not fully understood yet. The stimulus triggering the cascade of events that leads to restenosis comes from the injury of the vessel wall caused by balloon dilation and stent placement.⁽³⁾ Recoil of the vessel, neointimal proliferation, and early thrombus formation are the three different processes that are thought to play a role. The relative contribution of each of these depends on the type of injury. Coronary stenting virtually eliminates vessel recoil, and in-stent stenosis is largely due to neointimal proliferation. Growth factors and cytokines seem to be major stimuli for proliferation of smooth muscle cells after an artery is injured. Deposition of platelets, leukocyte infiltration, expansion of smooth muscle cells, deposition of extracellular matrix, and re-endothelialisation occur. The platelets release platelet-derived growth factor, transforming growth factor, epidermal growth factor, and thrombin, which stimulate the migration, growth and division of smooth muscle cells. The smooth muscle cells alter their phenotype from the contractile to a synthetic mode.⁽⁴⁾

Restenosis is not a random phenomenon, and certain patients are at increased risk of developing it. There are several clinical and lesion-related factors that explain part of the risk for restenosis. These factors include gender⁽⁵⁾, diabetes mellitus^(1;6;7), hypertension^(8;9), unstable angina⁽⁸⁾, severe coronary artery stenosis⁽⁸⁾, total occlusions⁽¹⁰⁾ and multivessel disease⁽⁷⁾, lesion length⁽¹¹⁾ lesions in the LAD⁽⁹⁾. However, a significant portion of this process cannot be predicted based on conventional risk factors.⁽⁹⁾

Various (pharmacological) strategies have proven to be disappointing in preventing the restenotic phenomenon. Other percutaneous coronary intervention techniques have been developed with varying efficacy. The introduction of the intracoronary stent has reduced the incidence of restenosis considerably. On the other hand in-stent restenosis still occurs and is more difficult to treat. The recently introduced drug-eluting stents have been shown to reduce restenosis rates more effectively. However, long-term follow up for most drug-eluting stents is still lacking and most research has only been done in selected population groups. Although these studies show a great reduction in the restenosis rate, drug-elut

ing stents have not eradicated restenosis completely.⁽¹²⁾

Genetic epidemiology may provide insights into the pathophysiology of coronary restenosis. Single nucleotide polymorphisms (SNPs), variants of a single nucleotide in DNA are the genetic markers mostly used in practice. Polymorphisms are DNA changes occurring in more than 1% of the population and the current hypothesis is that the human genome contains several polymorphisms that confer altered susceptibility to a disease. Stratification according to the genetic make-up can enable tailoring of interventional treatment to the individual patient. Furthermore, these insights might provide the basis for gene therapy, which in the case of restenosis may be locally delivered with relative ease.⁽⁶⁾

This review highlights the current insights into the genetic aspects of coronary restenosis. Polymorphisms evaluated in restenosis studies in humans will be summarized.

Methodological issues

To evaluate which genetic factors/ polymorphisms have been studied in the process of restenosis, a computer-based search was performed using MEDLINE and EMBASE databases with the keywords 'restenosis', 'PTCA', 'polymorphism', and 'genetics'. Also, references of the retrieved articles were screened for additional papers on the subject.

Currently, in establishing the restenosis rate after percutaneous coronary intervention, either angiographic and/or clinical criteria were used. Angiographic studies should either be analysed by quantitative computerized analysis methods or based on measurements by intravascular ultrasound. Both dichotomous and continuous variables can be deduced from quantitative computerized analysis. Restenosis is thought to be present if, after an initially successful percutaneous intervention, more than 50% luminal stenosis is present at follow-up angiography. Angiographic follow-up should be performed after 4 months, because the major degree of restenosis occurs between 3 and 4 months after the index intervention.

Clinical criteria are also used to establish restenosis rates. The major advantage of this approach is that clinical end-points reflect everyday practice with dichotomous variations. Currently used clinical criteria are target vessel revascularization, which represents clinically driven repeat procedures on the originally treated vessel, target site revascularization, which represents clinically driven repeat procedures on the originally treated site and target vessel failure, which

represents death or myocardial infarction not attributable to another vessel.⁽⁶⁾ Furthermore it is important to consider restenosis rates in the investigated population, because restenosis rates have decreased significantly compared to initial experiences.⁽⁷⁾

In addition, the genetic background among ethnic groups might be different with respect to numerous genes. For instance the prevalence of gene mutations and/or polymorphisms of Japanese patients cannot be extrapolated to Caucasian patients. In interpreting genetic epidemiological data, the ethnic background of the study population must therefore be considered.

Since stents almost abolish vascular recoil, other pathophysiological aspects of restenosis prevail. Therefore, in-stent restenosis should be considered as a separate phenotype than restenosis following plain balloon angioplasty.

The success rate of percutaneous coronary intervention is also dependent on the indication for the procedure. For instance, totally occluded vessels lead to restenosis more frequently than stable plaques. The clinical syndrome for which the interventional procedure was indicated should therefore be considered in the interpretation of the results.⁽⁶⁾

Results

In currently available reports, only a fraction of the genes that might be involved in coronary restenosis have been studied. The presently investigated polymorphisms, involve;

- The renin-angiotensin system
- Platelet aggregation
- The inflammatory response
- Matrix metalloproteinases
- Smooth muscle cell proliferation
- Lipids
- And oxidative stress and nitric oxide

Certain polymorphisms can be classified into more than one system. The important aspects of methodology discussed above will be reviewed systematically.

The renin-angiotensin system

The gene that encodes the angiotensin-converting enzyme (ACE) has been implicated as a genetic risk factor for restenosis after PTCA and has been studied

most extensively. Angiotensin II, the protein product of ACE, is a growth factor for vascular smooth muscle cells, dependent on interplay between the enhanced expression of other factors, including platelet-derived growth factor, transforming growth factor-beta, and fibroblast growth factor. The ACE level is one of the regulatory factors controlling the amount of neointima-formation after catheter interventions and may play an important role in the development of restenosis and especially in in-stent restenosis. The gene-encoding ACE is located on chromosome 17. The most studied variance is the insertion⁽¹⁾ or absence deletion (D) of 287 base pairs in intron 16. The genotypes are termed II, I/D, and DD, depending on homozygosity or heterozygosity for the insertion/deletion. The DD genotype of this polymorphism has been demonstrated to be linked to high plasma and tissue ACE levels.⁽¹³⁾

The association between the ACE DD-genotype and restenosis after PTCA is very controversial. After balloon angioplasty a Japanese study, of 82 patients with angiographically documented restenosis demonstrated that subjects with the DD genotype had a 4 times increased risk of restenosis. However, multivariate analysis was not performed, nor did the investigators mention differences in patient age or incidence of diabetes, both known risk factors for restenosis. Furthermore, the angiographic analysis was not quantitative but visual.⁽¹⁴⁾ An other study of 157 Italian patients also showed an increased risk for restenosis in D allele homozygotes (OR 7.46, 95% CI 0.97-57.42).⁽¹⁵⁾ However a different study of 511 patients, which were angiographically followed, did not find the ACE DD genotype to be an independent risk factor for restenosis after PTCA.⁽¹⁶⁾ Similar Samani et al. found no increased risk for restenosis in DD genotypes in the Subcutaneous Heparin and Angioplasty Restenosis Prevention (SHARP) trial. This study evaluated the effect of subcutaneous unfractionated heparin on restenosis in patients undergoing single vessel PTCA. The additional therapy did not prevent restenosis.⁽¹⁷⁾

The main finding of the study by Koch et al. is that the ACE gene I/D polymorphism is not associated with any appreciable increase in the risk for restenosis-driven adverse events in patients undergoing coronary stent placement. In this prospective study the ACE gene I/D genotype of 1850 consecutive patients with symptomatic coronary artery disease who underwent stent implantation was determined. The restenosis rate at the 6-month angiographic follow-up was 32.8% in patients with the II genotype, 34.0% for patients with the ID genotype, and 31.3% for patients with the DD genotype ($p=0.62$). One-year event-free survival was 77.7% in patients with genotype II, 75.2% in patients with genotype ID, and 75.5% in patients with genotype DD ($P=0.54$).⁽¹⁸⁾ Only one study linked both the ACE I/D polymorphism and plasma ACE levels to the occurrence of restenosis

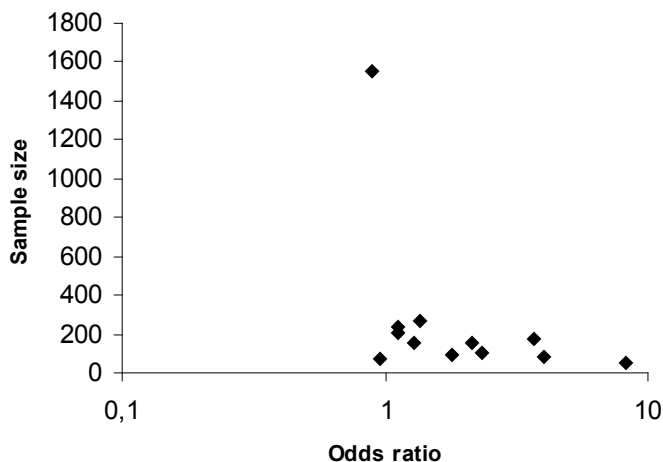
after coronary stenting. In this study patient selection was restricted to include only those without clinical factors and lesion characteristics associated with restenosis. Both the ACE level (RR 8.2, 95%CI 4.43-15.15) and the DD genotype (RR 2.75, 95%CI 1.51-5.03) were prognostic for the occurrence of restenosis. Of DD genotype carriers with an ACE level above 34U.l⁻¹, 62% had restenosis compared to 9.1% with low ACE levels. Thus, this study was the only one to affirm the assumption that the DD genotype causes neointima hyperplasia through high ACE levels. However, no effect on inhibition of ACE by chemical intervention on the restenosis rate has been demonstrated in the past.⁽¹⁹⁾

In a large prospective observational study by Hamon et al. 1010 consecutive white patients with symptomatic coronary artery disease who had successful PCI with stent implantation were prospectively studied. The influence of the ACE I/D genotype on the long-term risk of major adverse cardiac events (MACE) after PCI could also not be shown (MACE was reached in 35%, 37%, and 34% of patients with the DD, ID, and II genotypes, respectively, with no significant difference).⁽²⁰⁾

A meta-analysis has been performed on this subject, in which the authors conclude that a clinically significant association of the angiotensin-converting enzyme polymorphism with restenosis after PTCA in patients is unlikely. This meta-analysis provides evidence that the pooled estimate based on published literature, which favours an association, is distorted by publication bias. After correcting for publication bias, the overall OR of I allele carriers versus DD homozygotes was estimated to be only 1.15 (95% CI 0.98-1.32). Furthermore, in the absence of homogeneity between studies the best estimate is that of the study with the highest precision, which also yielded no evidence for an association (Figure 1).⁽¹³⁾

Another meta-analysis containing 16 studies (11 without stenting and 5 with stenting), showed that when the 16 studies were grouped by size, the combined odds ratios for restenosis in individuals with the DD genotype were 1.94 (CI 1.39-2.71) for the 11 studies with less than 100 cases, 1.33 (0.92-1.93) for the three studies with 100-200 cases, and 0.92 (0.72-1.18) for the two studies with more than 200 cases. Thus, this study shows that compared with other studies, larger and more rigorous studies show a weaker association between the angiotensin converting enzyme gene DD genotype and restenosis. Which can also be seen from the studies mentioned above.⁽²¹⁾

Other polymorphisms in the renin-angiotensin system were also examined. The angiotensinogen (AGT) polymorphisms T174M and M235T were studied in 511 patients with single or multi-vessel coronary artery disease. A total of 161 (31.3%)

Figure 1.

Funnel plot of all studies combined. The funnel plot provides a visual aid to detect publication bias. When no publication bias is present, the data should form a normal distribution. Because the distribution is skewed to the left, evidence for publication bias is present. Adapted from Agema et al.⁽¹³⁾

showed restenosis at follow-up angiography. This study provides evidence for an association of the AGT gene 235T variant with restenosis in PTCA patients. According to the results of a multifactorial analysis of covariance, the angiotensinogen gene M235T polymorphism contributed 1.4% to the total variability of the loss of lumen at follow-up angiography. The T174M polymorphism of the AGT gene was not associated with restenosis after PTCA. There is complete linkage disequilibrium between the AGT M235T and T174M polymorphisms. This linkage disequilibrium is characterized by the fact that all patients with the 174M allele also carry the 235T variant, but only a fraction of the patients with the 235T allele have the 174M allele. A study in a Japanese population found no association between the 235T variant of the AGT gene and restenosis after PTCA. The discrepancy between these two studies can be explained by ethnic differences between the study populations, or to differences in the number of patients enrolled in the two studies (less than 100 in the Japanese study).^(14;16)

Platelet aggregation

Platelets are thought to play an important role in restenosis and the platelet glycoprotein (GP) IIb/IIIa fibrinogen receptor may be critically involved. GPIIb and GPIIIa are polymorphic proteins, which can potentially influence both activation of the GPIIb-IIIa complexes or aggregation itself due to the dynamic

structure of these complexes. ⁽³⁾

The GPIIIa is a 90kDa integrin beta 3 subunit that is expressed on the cell surface. The gene coding for this protein has been mapped to chromosome 17. ⁽⁶⁾

GP IIb is a major GP of the human platelet plasma membrane, which together with GPIIIa forms the platelet fibrinogen receptor, the final pathway of platelet aggregation. The best-established variation of the fibrinogen receptor is the PIA polymorphism of GPIIIa, which is characterised by the presence of either a leucine (PIA1) or a proline (PIA2) at position 33 of the mature polypeptide. Recent studies have shown that PIA2 platelets have a lower aggregation threshold to certain agonists, raising the possibility that perturbations during PCI may more adversely affect PIA2 platelets. The finding in two large studies that patients with the PIA2 allele are at increased risk for stent thrombosis and restenosis supports this concept. ^(22;23) However in an Australian study of 208 subjects no differences in the PIA2 allele frequency between subjects with and without restenosis were found. ⁽²⁴⁾ The suggested hypothesis that carriers of the PIA2 allele have a more intense binding of fibrinogen and vitronectin and thus a higher risk of platelet-rich white thrombus formation, does expect a predominant risk for acute thrombosis over late restenosis. Recently developed therapies specifically inhibiting the IIb/IIIa receptor do reduce acute stent thrombosis but not in-stent restenosis rates. ⁽⁶⁾

The best known of the GPIIb polymorphisms is the diallelic system human platelet antigen-3 (HPA-3), characterised by HPA-3a, encoding isoleucine at position 843, and HPA-3b, encoding serine at this position. Unlike the GPIIIa, there is little information about the cardiovascular significance of the GPIIb polymorphism. In a study the presence of the HPA-3b allele was not associated with any increased risk of restenosis after coronary stent placement. ⁽²⁵⁾ An other large study involving 2178 consecutive patients also studied the impact of the GPIIb gene. Both angiographic and clinical end-points of restenosis were equally divided among the HPA-3a and HPA-3b allele carriers. Also, after adjustment for multiple variables, no statistically significant difference could be demonstrated among the different genetic subgroups. However, in these patient groups strong antiplatelet drugs were standard post procedural therapy, which might have influenced the impact of the genetic make-up of the patients. ⁽²⁵⁾

The GP Ia T807 polymorphism has also drawn attention owing to the role of GP Ia/IIa in platelet adhesion and activation. The glycoprotein Ia/IIa receptor complex (GPIa/IIa) is also known as integrin alpha 2 beta 1 or very late activation protein 2 (VLA-2) This glycoprotein is assumed to be a collagen receptor. The gene coding for the alpha 2 subunit of the VLA-2 receptor has been mapped to chromosome 5q23-q31. This gene is polymorphic within its coding

region. The carriage of the GP Ia T807 allele is however, not associated with an increased risk of restenosis or unfavourable late outcome following coronary artery stenting.⁽²⁶⁾

Inflammation

There is increasing evidence for an important role of inflammation in coronary artery disease. Cytokines play a pivotal role in regulating the inflammatory process. Till now research has been performed for some of the interleukines, which belong to the cytokine family and selectines. Also research has been done on growth factors, which are important mediators in the restenotic process.

Interleukins

Cytokines of the interleukin-1 (IL-1) family are central regulators in immunoinflammatory mechanisms; they regulate smooth muscle cell mitogenesis and extracellular matrix production. With these actions, IL-1 occupies a key place in the cascade of autocrine and paracrine mediators that promote restenosis after percutaneous coronary interventions. IL-1 has been established as a significant determinant of intimal hyperplasia and has been demonstrated to stimulate the thrombogenic response in endothelial cells as well as the production of endothelial-derived growth factor. A receptor antagonist, IL-1ra, counter regulates this cytokine. This is the product of a polymorphic gene (IL-1 receptor antagonist gene (IL-1RN)). Five polymorphic sites have been discovered in the IL-1RN: a variable number tandem repeat (VNTR) polymorphism in intron 2, and four single nucleotide polymorphisms, including one in exon 2, that show a high degree of linkage disequilibrium with the VNTR. These IL-1RN polymorphisms have been associated with altered production rates of IL-1ra protein. In view of the central role of IL-1 in regulation of the inflammatory response and the potent inhibitory effect of IL-1ra in this system, the hypothesis that the IL-1RN locus may contribute genetic risk in inflammatory diseases was proposed.⁽²⁷⁾ In a study by Kastrati et al., which included 1,850 consecutive patients who underwent coronary stent implantation, it was found that the presence of allele 2 in the IL-1ra gene was significantly associated with a lower risk of both angiographic and clinical restenosis. This association was even stronger in younger patients. A clear gene dose effect was found; the incidence of restenosis decreased with heterozygosity and even more so with homozygosity for the IL-1RN*2 allele. The presence of allele 2 has been shown to be associated with higher blood levels or higher monocyte production rates of IL-1ra but not in all populations and not when measured at IL-1RN RNA. Interleukin-1 receptor antagonist production increases with age and this may explain at least in part the more powerful ge

netic association in younger patients.⁽²⁷⁾

Also another study showed results that suggest an important association between IL-1RN*2 and protection from restenosis in individuals with single vessel disease.⁽²⁸⁾

Two single nucleotide polymorphisms in the interleukin-1 beta gene (IL-1B (-511) and IL-1B (+3954)) were investigated in 183 consecutive patients after successful PTCA. When analysed separately, none of the polymorphisms was associated with restenosis. However, when the IL-1B (-511) was combined with the IL-1RN VNTR genotype, a highly significant relationship was observed. Non-carriers of the two repeat allele of the IL-1RN VNTR (IL-1RN*2) who were heterozygous and homozygous for the IL-1B (-511) T allele exhibited a higher restenosis risk. In contrast, carriers of the IL-1RN*2 and the IL-1B (-511) T allele showed a significantly better outcome. This result gives evidence of the presumption that restenosis is a multifactorial vascular disease.⁽²⁹⁾

Another cytokine that has been investigated is interleukine 6 (IL-6). Il-6 promoter variant -174G>C was suggested in several studies to be associated with higher Il-6 levels post bypass and that the allele is associated with higher risk of CAD. However a study by Gomma et al. did not show that this variant has any significant influence on restenosis, although since the sample size was relatively small, a modest effect of this genotype cannot be ruled out.⁽³⁰⁾

The role of the other interleukines and/or their receptors is not yet clear and more research still has to be performed.

Selectins

Selectins, such as E-selectin, play a prominent role in the interactions between leukocytes, endothelial cells and platelets. An involvement of E-selectin in cardiovascular diseases is suggested by the fact that it is expressed only in activated endothelial cells and acts as an adhesive reactant. Following up regulation, the molecule is shed from the cell membrane and released as soluble E-selectin (sES) into the circulation. Increased concentrations of sES have been observed in subjects with acute myocardial infarction and peripheral vascular disease who developed restenosis after peripheral angioplasty. In two independent Caucasian study groups, the E-selectin 128Arg allele was more prevalent in CAD patients with post-angioplasty restenosis than in those without restenosis, independently of age and concomitant disorders. These findings are consistent with a role of E-selectin in the development of restenosis, reflecting a potential genetic aspect of an individual's risk to develop atherosclerotic complications.⁽³¹⁾

Growth factors

In addition to interleukins, cell adhesion molecules, including Mac-1 (CD11b/CD18), are key mediators of inflammatory reactions. Mutations of the CD18 gene can cause a leukocyte adhesion deficiency syndrome due to either diminished cell surface levels, or absence of CD18, or production of non-functional variants of CD18. A common SNP located in exon 11 of the CD18 gene, characterise by the presence of either cytosine or thymine at nucleotide position 1,323 and affecting codon 441, has been described. The association between this polymorphism was analysed in a large patient cohort (n=1,207) and it showed that the incidence of restenosis was lowest in the group of patients with the TT genotype (26.0%),

intermediate in patients with the CT genotype (31.7%), and highest in patients with the CC genotype (38.1%) (p=0.004).⁽³¹⁾ Thus, in essence, the 1323T allele was associated with an incremental reduction of restenosis. These data suggest an involvement of Mac-1 in the process of restenosis.⁽³²⁾

The CD14 receptor is a glycoprotein localized on the cell surface of all myeloid cells, especially on monocytes/macrophages. The stimulation of monocytes/macrophages by LPS induces overexpression of certain cytokines, complement components, coagulation factors, and others.⁽³³⁾ In a study by Zee et al. CD14 was found to be significantly associated with the incidence of restenosis (p=0.02).⁽³⁴⁾

Matrix Metalloproteinase

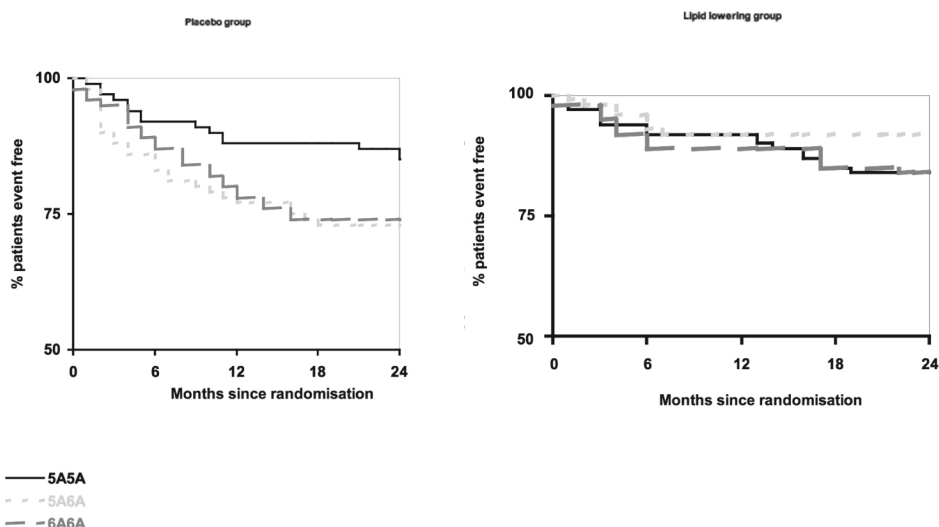
Matrix metalloproteinases (MMPs) form a family of zinc-dependent enzymes with proteolytic activity against connective tissue proteins such as collagen, proteoglycans, and elastin. Increased expression and activity of MMPs have been identified in various pathological processes, such as general inflammation, tumour metastasis, respiratory diseases, myocardial injury, vascular aneurysms, and remodelling. Because of their major significance in vascular remodelling, MMPs are suspected to play an important role in the pathogenesis of cardiovascular (CV) diseases, such as atherosclerosis and restenosis.⁽³⁵⁾ Especially stromelysin (MMP3) plays an important role in the process of connective tissue remodelling and wound healing. It is a proteoglycanase closely related to collagenase (MMP1) with a wide range of substrate specificities. It is a secreted metalloproteinase produced predominantly by connective tissue cells, it regulates the accumulation of extracellular matrix during tissue injury. Recently, a common functional variant in the promoter sequence of the MMP3 gene has been reported, in which one allele has a run of six adenosines (6A) while the other has only five (5A).⁽³⁶⁾ This gene has been mapped to a locus on 11q. In vitro studies and results of clinical trials suggest that, compared with other genotypes, individuals homozygous for

the 6A allele would have lower MMP3 levels in their arterial walls because of reduced gene transcription, which would therefore favour deposition of extracellular matrix and increase vessel constriction. A quantitative analysis of restenosis after conventional balloon coronary angioplasty showed that patients carrying the 6A6A MMP3 genotype had greater late luminal loss compared to patients with other genotypes. The mechanism of this association has been suggested by *in vitro* studies of promoter strength that showed a lower activity of the 6A promoter compared to that of the 5A allele in both cultured fibroblasts and vascular smooth muscle cells. Thus, subjects with the 6A6A genotype would be predicted to have lower activity of MMP3 in the vessel wall and in atherosclerotic lesions than those with other genotypes, and this lower proteolytic activity would result in less remodelling and thus faster deposition of extracellular matrix. This would result in faster progression of atherosclerosis, and this has been consistently seen in three angiographic studies of patients with coronary artery disease. An association between angiographic restenosis and 6A6A genotype was not detected in the group of patients who had had a stent implanted. Although this could be due to chance, the sample size of patients with and without a stent is of similar size, and this lack of association is in concordance with the suggestion that the processes occurring in patients with and without a stent is different.⁽³⁶⁾

One study confirmed that this polymorphism has no effect on restenosis after coronary stenting in a population of 226 patients.⁽³⁰⁾ In a subset of the REGRESS study the association of this genetic variant with restenosis after coronary intervention was studied. Coronary stenting was performed in a negligible portion of this population. A repeat revascularization procedure was performed more often in the 6A homozygotes and heterozygotes than in the 5A homozygotes. The 6A homozygotes appeared to have a lower revascularization rate when treated with pravastatin, 15% vs. 40% when on placebo. Thus, this genetic variant is associated with a higher risk on restenosis, which is reduced by pravastatin. It might be postulated that pravastatin influences the remodelling process as well (Figure 2).⁽³⁷⁾

Smooth muscle cell proliferation

The Pro7 allele of the preproNPY gene affects the plasma levels of human neuropeptide Y, a potent mitogen of vascular smooth muscle cells. In a population of 1,850 consecutive patients with symptomatic coronary artery disease undergoing coronary stent implantation were enrolled in a study that featured angiographies at 6 months and genotype determination. The Leu7 to Pro7 polymorphism of the preproNPY gene is not associated with angiographic restenosis or adverse clinical events after stent placement in coronary arteries.⁽³⁸⁾

Figure 2.

Clinical event free survival curves for each stromelysin genotype solid line: 5A5A; dotted line: 5A6A, and hatched line: 6A6A in A the placebo and B the pravastatin groups, adjusted for baseline medication. Adapted from de Maat et al.⁽³⁷⁾

Gi signalling is related to vascular smooth muscle proliferation. In addition, Gi signalling plays a distinct role in agonist-induced platelet activation since Gi and Gq signalling are required for agonist-induced platelet aggregation. However, Gbeta3: C825T polymorphism does not seem to influence the mechanisms leading to restenosis and thrombosis following coronary stenting.⁽³⁹⁾ In another study of 562 patients, the 825C/T SNP was also tested, but no influence of this polymorphism on the incidence of restenosis after coronary stenting was found.⁽³⁾

Lipids

Plasma lipids may play a role in restenosis, although no direct association between cholesterol level and the occurrence of restenosis has been demonstrated. Apolipoprotein E (apo E) plays a key role in cholesterol and triglyceride metabolism. It is a constituent of chylomicrons and very low-density lipoprotein (VLDL) remnants and acts as a ligand for their receptor-mediated uptake and clearance by the liver. The human apo E gene is located on chromosome 19. The gene is polymorphic with three common alleles encoding the major plasma apo E isoforms, apo E2, apo E3 and apo E4 respectively. These proteins differ by amino acid substitutions at one or both of positions 112 and 158 of the 299

amino acid proteins, the latter being the principal residue of the binding domain for the apo E receptor. The apo E₃ polypeptide is the most common isoform. In comparison to it, the apo E₄ isoform is associated with higher and the apo E₂ isoform with lower plasma cholesterol levels.⁽⁴⁰⁾ Van Bockxmeer et al. found that of 195 Australian patients treated with elective angioplasty of previously untreated native coronary artery, 69 (35%) developed restenosis. Carriers of the apo E₄ allele had a ten-fold increased risk of restenosis (OR 10.0, 95%CI, 1.2-90). No interaction with prescribed medication could be established. Surprisingly, the mean level of LDL cholesterol was higher in the group without restenosis (4.08 vs. 3.68 mmol.l⁻¹, P<0.02) The study also reported that the apo E₄ allele may interact synergistically with the deletion (D) allele of the angiotensin converting enzyme (ACE) gene to increase the risk sixteen-fold. Apo E₄ could potentially influence restenosis through several other mechanisms. It could act directly by binding to key extracellular or cellular components in the vascular wall involved in the restenotic process. Alternatively, it could act by modulating the function of monocytes/macrophages, which are present in the atherosclerotic plaque and are an important source of vessel wall cytokines. Apo E is known to be synthesized and secreted by such cells.⁽⁴¹⁾ Samani et al. could not confirm these results in an English study, where follow-up angiography was performed 4 months after PTCA. Of 265 eligible subjects, blood for genotyping was available in 231 patients. They were unable to demonstrate a significant effect of either apo E₄ carrier status or homozygosity on restenosis. They were also unable to demonstrate any interaction between the ACE D and apo E₄ alleles.⁽⁴⁰⁾ A study by Flork et al. including exclusively women did show a correlation between the apolipoprotein apo E₄ allele and high LDL cholesterol and Lp(a) levels but no association between the apo E₄ allele and restenosis was identified.⁽⁴²⁾

A study in a selected patient population by Tada, did suggest that the E₄ allele is associated with a higher restenosis rate after PTCA.⁽⁴³⁾ Clearly the studies that have been published on the apolipoprotein E polymorphism in relation to restenosis have contradictory results. Sample size might be one explanation for this result, considering that apolipoprotein E₄ homozygosity is rare, occurring in 3% of the population.⁽⁶⁾

Lp(a) contains apolipoproteine(a), which is a structural homologue of plasminogen and competes with it for binding sites. It also acts by increasing plasminogen activator inhibitor-1 expression, thus, interfering with endothelial cell and circulating plasmin generation. Lp(a) is involved in lipid metabolism, the coagulation and fibrinolytic systems, and the stimulation of smooth muscle cell proliferation⁽⁴⁴⁾ Lipoprotein(a) is a powerful genetic risk factor for CAD. Elevated Lp(a) levels were not associated in a study by Wehinger et al. with an adverse one-year

clinical and angiographic outcome after stent placement.⁽⁴⁵⁾ Another study also showed that Lp(a) and apo(a) polymorphism do not appear to be reliable markers of restenosis in patients with stent implantation.⁽⁴⁶⁾

Oxidative stress and nitric oxide

The importance of oxidative stress and reactive oxygen in humans and animals has been well described. Accordingly, differences between persons in their level of antioxidant protection may influence their risk of developing restenosis. Haemoglobin is an important mediator of oxidative tissue damage that is released from red blood cells at sites of vascular injury. Haptoglobin is a serum protein that serves as an antioxidant by virtue of its ability to bind to haemoglobin and prevent haemoglobin-mediated oxidative tissue damage. In humans, there are two general classes of alleles for the haptoglobin (HP) gene, designated 1 and 2. The different haptoglobin phenotypes appear to differ in their antioxidant capacity, with the Hp1 protein being the most superior antioxidant. The risk of developing restenosis was greater in subjects with 2 Hp2 alleles than in those with 1 Hp 2 allele or no Hp 2 alleles. Similar results were also found in another study, which also demonstrated that patients homozygous for the Hp1 allele were found to have a significantly lower rate of restenosis. Moreover a graded risk relation to the number of Hp 2 alleles was demonstrated.⁽⁴⁷⁾

Endothelial nitric oxide synthase (eNOS) catalyses the formation of nitric oxide which has vasodilatory, antithrombotic, anti-inflammatory and antiproliferative properties.⁽⁴⁸⁾ Synthesis of nitric oxide from the amino acid L-arginine is catalysed by nitric oxide synthase families. Three isoforms of nitric oxide synthase have been identified so far. Reduced synthesis of nitric oxide may promote the proliferation of vascular smooth muscle cells and induce in-stent restenosis. The 298Asp variant has been shown to be functional as it results in protein that is more susceptible to proteolytic cleavage, and thus leads to lower eNOS levels. The Asp allele has been shown to be associated with coronary artery disease and myocardial infarction. The missense Glu298Asp variant in exon 7 of the endothelial nitric oxide synthase gene contributes to endothelial dysfunction. One study provides evidence that the missense Glu298Asp variant is an independent risk factor for in-stent restenosis.⁽⁴⁹⁾ A different study also showed a significant relation to Glu298Asp and $-786T>C$ and in-stent restenosis. The $-786T>C$ polymorphism, which has been reported to be associated with coronary artery spasm, is also thought to be functional. The $-786T>C$ reduces eNOS gene promoter activity, and a lower promoter activity would also lead to less NO production with the consequence of more smooth muscle cell proliferation and restenosis.⁽³⁰⁾ Another single nucleotide polymorphism, the 894G/T, located in exon 7 of the

eNOS gene, was investigated in a study, which included 1850 Caucasian patients with CAD who were treated with stent implantation. TT patients showed no significant increase in the risk for angiographic restenosis (OR; 1.11, 95%CI 0.78-1.56; $p=0.56$) and target vessel revascularization (OR 1.21, 95%CI 0.82-1.78; $p=0.34$).⁽⁴⁸⁾

Combined genetic variations

Restenosis after PTCA procedures is assumed to be a multifactorial and multigenetic process. It would therefore be appropriate to investigate the impact of different genetic variations together. Nowadays, the development of well-designed DNA-microarrays with the possibility of testing some 50 or 60 polymorphisms with one DNA sample facilitates these studies. One study has already been published, while using this technique. This study by Zee et al. determined genotypes for 94 SNPs representing 62 candidate genes, in a prospectively assembled cohort of 342 cases and 437 controls. They used a customized coupled-logistic regression procedure accounting for both additive and interactive effects and identified seven SNPs in seven genes that together, showed a statistically significant association with restenosis incidence ($p<0.0001$), accounting for 11.6% of overall variance observed. Among them were candidate genes for cardiovascular pathophysiology, inflammatory response and cell-cycle control; apolipoprotein (APOC3 C1100T); monocyte differentiation antigen CD14 (C (-260) T); endothelial nitric oxide synthase (NOS3 E298D); tumor necrosis factor receptor 1 (TNFR1 A845G); tumor suppressor protein P53 (TP53 P72R); p53-associated protein (MDM2 N1aIV site) and cystathionine-beta-synthase (CBS I278T).⁽³⁴⁾

The second study that tested for possible associations between candidate gene polymorphisms and the risk of restenosis after PTCA was performed by Völzke et al. They did however not make use of the DNA-microarrays but tested 10 different polymorphisms in different genes in a study population of 511 patients. They looked at beta-fibrinogen, glycoprotein IIIa; factor V Leiden, tumor necrosis factor alpha, interleukin-1A and 1B, methylenetetrahydrofolate reductase and endothelial nitric oxide synthase. However in that study no association between one of those polymorphisms and restenosis were observed. However this study showed several limitations, which could contribute to the negative outcome. These include; limited statistical power, incomplete follow-up and deviations of the genotype distribution of several gene polymorphisms from the Hardy-Weinberg equilibrium in the study population.⁽⁵⁰⁾

One of the problems that occur with these kinds of studies is the issue of correction for multiple testing. That is why these kinds of studies should probably be regarded as an exploratory study and the results must be interpreted accordingly.

They can reveal genetic polymorphisms that warrant testing in further prospective studies with sufficient power.

Conclusion

New insights into the pathophysiology of restenosis after coronary interventions have been elucidated by genetic studies. Functional variants of genes involved in different processes have been found. These include; the renin-angiotensin system, platelet aggregation, the inflammatory response, matrix metalloproteinases, smooth muscle cell proliferation, lipids and oxidative stress and nitric oxide. The results of these studies must be interpreted with great caution as numerous different extrinsic factors are observed in these studies, which might influence genetic contribution to restenosis differently. Furthermore many of these studies are of relatively small sample size and exhibit wide confidence intervals. Initial enthusiasm of results from smaller studies frequently has resulted in disappointment from larger ones. On the other hand, one must take into account that the process of restenosis is a multifactorial one and it is likely that multiple genes are involved. Thus, relatively small odds ratios relating to single gene contribution to restenosis might be of paramount importance when encompassed in the overall picture. The identification of gene polymorphisms associated with restenosis and in-stent restenosis can provide possible targets for gene therapy. (6) Gene transfer may offer a new treatment option for cardiovascular diseases. Experimental studies have demonstrated successful arterial gene transfer by using several genes and vectors, and phase I/II trials in patients with severe vascular diseases using different therapeutic genes (VEGF, FGF, E2F decoy) have been reported. Adenoviruses have also shown their potential in vascular gene transfer. ⁽⁵¹⁾

The investigated polymorphisms described above are only a few considering the complex mechanisms underlying the process of restenosis. Candidate genes encoding proteins involved in the development of restenosis, like platelet-derived growth factor, transforming growth factor, insulin-like growth factor, transforming growth factor and other interleukines still have to be evaluated in the near future. Microarray techniques that enable the testing of multiple genes will greatly speed-up genetic research and may give more insight in the process of restenosis. Stratification according to genetic make-up can enable tailoring of interventional treatment to the individual patient.

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