Clinical research

The 5A/6A polymorphism of the stromelysin-1 gene and restenosis after percutaneous coronary interventions

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Aims Matrix metalloproteinase stromelysin-1 has been implicated in the process of exaggerated lumen re-narrowing after primarily successful interventions in coronary arteries. We examined the possibility that the 5A/6A promoter polymorphism of the stromelysin-1 gene is associated with restenosis after stenting or percutaneous transluminal coronary angioplasty (PTCA).

Methods and results The study included 3333 consecutive patients with symptomatic coronary artery disease who were treated with stent implantation (n=2857) or PTCA (n=476). Primary end-point was angiographic restenosis, defined as ≥50% diameter stenosis at 6-month follow-up angiography. Restenosis rates were 28.1%, 27.8%, and 29.5% in carriers of the stromelysin-1 genotypes 5A5A, 5A6A, and 6A6A, respectively (P=0.71). The incidence of death or myocardial infarction and the need for revascularization at the site of the intervention due to symptoms or signs of ischaemia in the presence of angiographic restenosis were not significantly different between the genotype groups at 1 year. Separate analysis of the patients who underwent stenting and the patients who were treated with PTCA did not indicate the existence of a treatment type-related association between the 5A/6A polymorphism and restenosis.

Conclusion Our data strongly suggest that the 5A/6A polymorphism of the stromelysin-1 gene is not related to angiographic restenosis or the 1-year clinical outcome after interventions in coronary arteries.

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KEYWORDS
Stromelysin-1;
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Introduction

Stent implantation and percutaneous transluminal coronary angioplasty (PTCA) are established treatments of coronary artery disease.1–3 Coronary interventions inevitably cause mechanical injury of the arterial wall and elicit wound healing reactions.4,5 In 20% to 50% of the cases, exaggeration of the normal reparative process ensues which leads to significant re-narrowing (restenosis) of the vessel lumen.6 Balanced degradation and synthesis of extracellular matrix is essential for normal wound healing of injured arteries.7,8 The extent of extracellular matrix breakdown and accumulation is tightly regulated by the interplay between matrix metalloproteinases (MMPs) and their endogenous antagonists, the tissue inhibitors of metalloproteinases.7,8 In addition, timely conversion of MMPs from their proenzyme form to the mature form is an important control mechanism for matrix turnover.7,8 Inappropriate reorganization of the matrix scaffold of the artery, due to aberrant
matrix degradation, may result in constrictive artery remodelling.\textsuperscript{9,10} In this regard, examination of coronary target lesions by intravascular ultrasound suggested that restenosis after PTCA is predominantly the consequence of a decrease in external elastic membrane cross-sectional area, a remodelling process which results in shrinking of the artery and reduction of the arterial lumen.\textsuperscript{11,12} Alternatively, degradation of the internal elastic lamina by MMPs may facilitate migration of vascular smooth muscle cells (VSMCs) from the media to the intima, followed by neointima formation due to VSMC proliferation and accumulation of extracellular matrix.\textsuperscript{10} In line with this possibility, lumen narrowing due to neointimal hyperplasia, rather than constrictive remodelling, was observed to be the major mechanism for restenosis after stenting in coronary arteries.\textsuperscript{13–15}

The MMP stromelysin-1, also known as MMP-3, may play a critical role in the development of restenosis after coronary interventions. Stromelysin-1 catalyses the breakdown of extracellular matrix macromolecules, including proteoglycans, different types of collagen, fibronectin, and laminin, and facilitates the conversion of other MMPs, such as collagenase-1 (MMP-1), collagenase-2 (MMP-8), matrilysin (MMP-7), and 92-kDa gelatinase (MMP-9), from their inactive to their proteolytically active forms.\textsuperscript{16–20} Involvement of stromelysin-1 in restenosis was suggested by the finding that human atherosclerotic plaques, which are the target of coronary interventions, contained elevated levels of stromelysin-1 and matrix-degrading activity.\textsuperscript{21} Further support for this possibility came from the observations that VSMCs and lipid-rich macrophages of human atherosclerotic plaques contained stromelysin-1 mRNA and that cytokine-stimulated human VSMCs and macrophage foam cells from experimental atheroma produced stromelysin-1 and exhibited activated forms of MMPs.\textsuperscript{22–24} A role for stromelysin-1 in restenosis was also suggested by the findings that (i) mechanical injury of rabbit VSMCs was associated with induction of stromelysin-1 mRNA and (ii) migration and proliferation of VSMCs and neointima formation after vessel wall injury of rat carotid arteries was substantially inhibited by antisense oligonucleotides to stromelysin-1 mRNA.\textsuperscript{25,26}

The human stromelysin-1 gene contains a common promoter polymorphism, characterised by runs of 5 or 6 adenosines, located at nucleotide positions 1171–1175 or 1171–1176, respectively, upstream from the start site of transcription.\textsuperscript{17,28} Transfection experiments with tissue culture cells showed that the promoter with the 5A sequence has a higher activity than the promoter with the 6A motif, probably attributable to preferential binding of a putative transcriptional repressor protein to the promoter containing the 6A sequence.\textsuperscript{29}

Due to its important function in extracellular matrix turnover, stromelysin-1 may contribute to neointima formation and/or arterial remodelling after coronary interventions and the 5A/6A polymorphism may represent a genetic marker for this function. We examined this possibility in a large series of patients with coronary artery disease who were treated with stenting or PTCA.

### Methods

#### Patients

The study included 3333 consecutive Caucasian patients with symptomatic coronary artery disease who were treated with stent implantation (2857 patients) or PTCA (476 patients) at Deutsches Herzzentrum München and 1. Medizinische Klinik, Klinikum rechts der Isar der Technischen Universität München. The patients were scheduled for follow-up angiography at 6 months. The protocol of PTCA, stent placement and post-intervention therapy is described in detail elsewhere.\textsuperscript{30,31} Different stent types were used: ACS RX Multi-Link (Guidant, Advanced Cardiovascular Systems, Santa Clara, California, USA) (31.2%), JOSTENT (JOMED International AB, Helsingborg, Sweden) (17.7%), ACS Multi-Link RX Duet (14.2%), Medtronic AVE (Medtronic AVE, Santa Rosa, California, USA) (13.2%), BX Velocity (Cordis Corp., Miami, Florida, USA) (12.6%), and others (11.1%). Post-procedural therapy consisted of aspirin (100 mg twice daily indefinitely) and ticlopidine (250 mg twice daily for 4 weeks). Patients who were considered at a higher risk for stent thrombosis received additional therapy with the glycoprotein IIb/IIIa blocker abciximab. All patients gave written informed consent for the intervention, follow-up angiography and genotype determination. The study protocol conformed to the Declaration of Helsinki and was approved by the institutional ethics committee.

#### Determination of the stromelysin-1 genotypes

Genomic DNA was extracted from peripheral blood leukocytes using commercially available kits (Qiagen, Hilden, Germany, and Roche Applied Science, Mannheim, Germany). Genotype analysis was performed with allele-specific fluorogenic oligonucleotide probes in an assay combining the polymerase chain reaction (PCR) and the 5′ nuclease reaction (TaqMan technique; Applied Biosystems, Darmstadt, Germany). Oligonucleotide primers 5′-GCCACCTGGCTAAAAGACATT-3′ and 5′-GTGGCCAATACTTCCCTGTATT-3′ were used to amplify a 114-bp portion of the upstream region of the stromelysin-1 gene (nucleotide positions –1127 to –1240, according to the numbering of Quinones et al.\textsuperscript{28}). The sequence of the probe specific for the 5A allelic copy was 5′-FAM-AAGACATGTTTTTCCCCCCTACCAAA-3′ and the sequence of the probe specific for the 6A allelic copy was 5′-VIC-AAGACATGTTTTTTCCCCCCCCATCAA-3′. The sequences of the probes were complementary to the promoter sequence of the stromelysin-1 gene reported by Quinones et al.\textsuperscript{28} The runs of five and six thymines (T; underlined) represent the 5A and 6A motifs, respectively. FAM, i.e. 6-carboxy-fluorescein, and VIC (proprietary dye of Applied Biosystems) were the fluorogenic dyes used to accomplish allelic discrimination. The 2-step thermocycling procedure consisted of 40 cycles of denaturation at 95 °C for 15 s and primer annealing and extension at 62 °C for 1 min. The accuracy of the genotyping results obtained with the TaqMan method was examined by nucleotide sequence analysis of a limited set of DNA samples (n=10); DNA sequencing was done at an independent institution (GATC GmbH, Konstanz, Germany). As a control for correct sample handling and genotype data acquisition, genotyping was repeated for 20% of the samples using DNA prepared separately from the original blood sample. Genotype determination was done without knowledge of angiographic and clinical results.

#### Angiographic assessment

Quantitative computer-assisted angiographic analysis was performed off-line on angiograms obtained just before and
Study end-points and definitions

The primary end-point of the study was angiographic restenosis defined as a diameter stenosis of \( \geq 50\% \) at 6-month follow-up angiography. Secondary end-points were the incidence of death or myocardial infarction and the need for target vessel revascularization (TVR) (PTCA or aortocoronary bypass surgery) due to symptoms or signs of ischemia in the presence of angiographic restenosis at the site of the intervention over 1 year. The diagnosis of acute myocardial infarction was based on the criteria applied in the Evaluation of Platelet lib/lla Inhibitor for Stenting trial (EPISTENT): new pathological Q-waves or a value of creatinine kinase or its MB isoenzyme more than three times the upper limit.\(^3\) Creatine kinase levels were determined systematically over 48 h after the intervention. The follow-up protocol included a phone contact or a medical visit at the outpatient clinic at 30 days and between 9 and 15 months after stent placement or PTCA and a control angiography at 6 months. Clinical events were assessed on the basis of the information provided by hospital readmission records, referring physician, or phone interview with the patient. For all patients who presented cardiac symptoms during the interview, at least one clinical and electrocardiographic evaluation was performed at the outpatient clinic or by the referring physician.

Statistical analysis

Discrete variables are expressed as counts or percentages and were compared with the chi-square or Fisher exact test, as appropriate. Continuous variables are expressed as mean±standard deviation and were compared by means of the unpaired, two-sided t-test or analysis of variance for more than two groups. The sample size of the study was chosen to provide the analysis with at least 80% power, based on the following assumptions: (i) the proportion of the patients with the 6A6A genotype is 25%; (ii) the overall restenosis rate is 30%; (iii) the restenosis rate among the patients with the 6A6A genotype is increased by 20% compared to the patients carrying the 5A allele (assumed restenosis rates: 35% vs 29%). For an \( \alpha \) error of 0.05, 2500 patients with follow-up angiography were required. To accommodate for an expected loss of 20–25% to follow-up angiography, 3333 patients were included in the study. We tested for independent association between the 5A/6A polymorphism and angiographic restenosis in a multivariate analysis (multiple logistic regression) which included as potentially confounding factors: age, gender, arterial hypertension, diabetes mellitus, current tobacco smoking, hypercholesterolaemia, acute myocardial infarction, unstable angina pectoris, previous myocardial infarction, previous bypass surgery, multivessel disease, left ventricular ejection fraction, target coronary vessel, lesion complexity, restenotic lesion, chronic occlusion, reference diameter, lesion length, diameter stenosis before stenting, and type of intervention. In the multivariate analysis of the subgroup of patients with stenting, the types of stents were included as additional factors. Adjusted odds ratios and 95% Wald confidence intervals were calculated on the basis of the multiple logistic regression models. Statistical analyses were performed using S-Plus software (Mathsoft Inc, Seattle, Washington, USA). A \( P \) value of <0.05 was considered statistically significant.

Results

The results of the test series, i.e. parallel DNA genotyping with the TaqMan method and sequence analysis, were fully corresponding, which demonstrated the accuracy of genotyping with the TaqMan system. In our study population of 3333 patients, the stromelysin-1 genotypes 5A5A, 5A6A, and 6A6A were present in 801 (24.0%), 1667 (50.0%), and 865 (26.0%) subjects, respectively. The genotype distribution was in agreement with the Hardy–Weinberg equilibrium (\( P=0.97 \)). Baseline clinical characteristics of the patients, lesion variables before coronary intervention, and procedural parameters were not significantly different between the genotype groups (Table 1).

Primary end-point analysis

Six-month follow-up angiography was performed in 2667 patients (80.0%). The rates of restenosis did not significantly differ between the groups: 28.1%, 27.8%, and 29.5% in carriers of genotype 5A5A, 5A6A, and 6A6A, respectively (\( P=0.71 \); Table 2). Continuous measures of angiographic restenosis, minimal lumen diameter, percent diameter stenosis, and late lumen loss, were not markedly different between the genotype groups (\( P=0.33 \); Table 2). We examined the influence of potentially confounding factors on the relationship between the 5A/6A polymorphism and angiographic restenosis (see Methods for details). After adjustment for these factors, multivariate analysis (multiple logistic regression) did not reveal an association (\( P=0.29 \)). The adjusted odds ratio was 1.05 (95% CI 0.80–1.38).

Secondary end-point analysis

Complete 1-year clinical follow-up data were available for all patients, irrespective of the presence or absence of control angiography. The incidence of death or myocardial infarction and the need for TVR at the site of the intervention due to symptoms or signs of ischemia in the presence of angiographic restenosis were not significantly different between the genotype groups (\( P=0.24 \) and \( P=0.44 \), respectively) (Table 3).

Subgroup analysis

We separately examined the putative effect of the 5A/6A polymorphism on the angiographic and clinical outcomes in patients treated with stenting (\( n=2857 \)) and in patients who underwent PTCA (\( n=476 \)). Genotypes 5A5A, 5A6A,
and 6A6A were similarly distributed in the patient subgroups: 23.9%, 50.1%, and 26.0% in the group with stenting and 24.8%, 49.4%, and 25.8% in the group with PTCA, respectively. The genotype distribution of either group was in Hardy–Weinberg equilibrium ($P > 0.79$). Angiographic follow-up examination was done in 2286
patients (80.0%) of the group with stenting and in 381 patients (80.0%) of the group with PTCA. Restenosis was observed in 26.4% of the patients with stenting and 39.9% of the patients with PTCA (P<0.0001). However, as shown in Table 4, the rates of restenosis were not significantly different between the genotype groups of the patients with stenting (P=0.81) and of the patients with PTCA (P=0.73). Regarding the continuous measures of angiographic restenosis, we also did not find significant differences in the genotype distributions of the two patient groups (P>0.20; Table 4). Because different types of stents were used, we examined the impact of the 5A/6A polymorphism on restenosis in relation to the stent types. With none of the stent types did we detect an association between the polymorphism and restenosis (P>0.46) (Table 5). In addition, we did not observe a significant interaction between stent type and genotype with respect to the risk of restenosis (P value for interaction=0.86). We also examined the influence of potentially confounding variables on the relationship between the 5A/6A polymorphism and restenosis among the patients with stenting and the patients with PTCA. With the multivariate models described in the Methods section, we did not find such an association in the group with stenting (P=0.84) or the group with PTCA (P=0.40). The adjusted odds ratios were 1.03 (95% CI 0.78–1.36) in the patients treated with stenting and 1.31 (95% CI 0.70–2.44) in the patients treated with PTCA. Finally, the incidence of death or myocardial infarction and the need for TVR due to angiographic restenosis was not significantly different among the genotype groups of the patients with stenting or PTCA at 1 year (P>0.15) (Table 6).

Discussion

The main result of the study was that the 5A/6A promoter polymorphism of the stromelysin-1 gene was not associated with angiographic restenosis in patients undergoing coronary interventions. Separate analysis of the patients who underwent stenting and the patients who were treated with PTCA did not indicate the existence of a treatment type-related association between this polymorphism and restenosis.

Regarding angiographic restenosis, the primary endpoint of the study, we consider it unlikely that the results are based on chance because this study included a large number of consecutive patients (n=3333), 80% of whom underwent 6-month angiographic follow-up. Operator-related biases were greatly excluded: physicians who performed follow-up angiography or examined angiograms were blinded to the genotype data and workers...
who determined the genotypes were unaware of patients’ clinical and angiographic data. The genotype distribution of our study population was similar to findings in unrelated clinical settings: in a population of 485 French patients with symptomatic coronary artery disease, the genotype distribution was 23.9% 5A5A, 51.1% 5A6A, and 24.9% 6A6A, and in a group of 494 patients, without vascular disease, the genotype distribution was 24.1% 5A5A, 50.2% 5A6A, and 25.7% 6A6A. In a group of 515 male German individuals, Humphries et al. did not find an association of the 5A/6A polymorphism with angiographic restenosis or continuous measures of restenosis, e.g. late lumen loss, in the patients treated with stenting (n=198). In the group of patients with PTCA (n=287), Humphries et al. observed significantly higher late lumen loss (P=0.012) and diameter stenosis (P=0.012) among the patients with the 6A6A genotype compared to the carriers of the 5A allele (5A5A or 5A6A genotype). Regarding the patients with PTCA, our results are in conflict with the findings of Humphries et al.; in contrast to their report, our data indicated that late lumen loss (P=0.46) and diameter stenosis (P=0.20) were not significantly different between the genotype groups (Table 4). We do not know the reason for the discrepancy between the results we achieved and the data provided by Humphries et al. Differences in study design and baseline characteristics of the patients, such as age and gender distribution, offer possible explanations.

**Limitation of the study**

We achieved a follow-up angiography rate of 80%. Previous trials on restenosis have also shown the impossibility of achieving higher rates because of patient refusal in an inevitable proportion of cases. However, the lack of an association between the 5A/6A polymorphism of the stromelysin-1 gene and restenosis in the entire study population and the patient subgroups was also evident from the clinical outcomes, such as the need for re-intervention.

**Conclusion**

We did not find an association between the 5A/6A polymorphism of the stromelysin-1 gene and the risk of angiographically defined restenosis after interventions in coronary arteries. This result was true for the entire study population and for subsets of patients grouped according to the type of treatment, stent implantation or PTCA. Despite these findings, our results do not negate the potential role of stromelysin-1 in the initiation or progression of restenosis.

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**References**


